Degradation Of Activated Sludge UnderDifferent Redox Conditions And Degradability Of Endogenous Residues For Sludge Reduction

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Summary

Processes that lead to sludge reduction (i.e., a reduction of the overall amount of sludge produced in a system) were studied under conditions that are typically relevant for smaller wastewater treatment plants. Small treatment plants are often infrequently serviced making separate sludge treatment and valorization technically, economically, and environmentally not advantageous. In this thesis, the applied conditions for in-situ sludge reduction were exposure of sludge to alternating redox conditions and long solids retention times (SRTs). The objective was to improve understanding of the mechanisms leading to sludge reduction under these conditions (alternating redox and long SRT) and to quantify the rates of different sludge reduction processes. The experimental approach consisted of monitoring organic fractions from activated sludge (a.k.a. secondary sludge) under different redox conditions and over long SRTs and comparing that to systems response in terms of sludge reduction, such as observed sludge yield (Y_{obs}) or organic solids degradation rate. The following sludge fractions were monitored: mixed liquor volatile suspended solids (VSS) and its constituents in terms of the cellular biomass (quantified by intracellular adenosine tri-phosphate (ATP)), extracellular polymeric substances (EPS) and the endogenous residues. The sludge fraction of unbiodegradable influent particulate organics was not the focus of the investigations in order to concentrate the research efforts on the other fractions.

The processes under alternating redox conditions were studied in a wastewater treatment system that consists of a conventional activated sludge process (AS) coupled to a side-stream reactor (SSR) in the return activated sludge line (the overall system is referred to as AS-SSR system). The AS-SSR system had been used to study sludge degradation processes in previous investigations and the system is also implemented at full-scale facilities. Reports from full-scale systems showed that sludge reduction performance of these systems is variable and that this variability is not sufficiently predictable with current understanding. Thus to improve the understanding of the sludge reduction processes in such a system is clearly relevant for applications. In different experimental phases the side-stream reactor (SSR) was operated under fermentative (oxido-reduction potential at -380 mV) and scarce aeration (oxido-reduction potential at -250 mV) to expose sludge to different redox conditions inside of the AS-SSR system. In a third experimental phase, the SSR was operated under aerobic conditions. It was

found that the organic sludge constituents of cellular biomass and EPS were degraded fastest under aerobic conditions, as well as the overall organics in terms of VSS (VSS degradation rate was 2.2–4.1 times faster under aerobic condition than under scarce aeration condition). The highest sludge reduction performance under aerobic conditions are the plausible explanation for lowest Y_{obs} that was achieved generally with the aerobic operation of the SSR (Y_{obs} was up to 27% lower with aerobic operation of the SSR as compared to oxygen limited operation). The increase in SRT was shown to reduce Y_{obs} in general under all three operation modes of the SSR (aerobic, scarce aeration and fermentative). It is important to underline the influence of SRT on Y_{obs} since this factor was sometimes not sufficiently discussed in sludge reduction literature.

Application of very long SRT in a wastewater treatment system is beneficial only if organic sludge fractions that are traditionally considered as inert organics, such as endogenous residues, are degradable. Degradability of endogenous residues was shown for secondary sludge that was produced on municipal primary effluent in this thesis. The first order degradation rate for endogenous residues ($b_{XU,E}$) was 0.7 and $1.1 \cdot 10^{-3}$ d^{-1} in intermittent aerated and full aerated batch digestion, respectively, at ambient temperature (around 20 °C) between day 100 and 380 of digestion. The degradation of endogenous residues was thus shown for real active sludge under conditions that can be found in very small, decentralized wastewater treatment facilities, these conditions are ambient temperature and very reduced contact of the sludge with active biomass. But the process of endogenous residues degradation was very slow, approximately seven times slower than previously reported in literature). Apparent degradation of endogenous residues was found in AS-SSR systems suggesting that excess sludge production prediction is improved by considering this process. But the determined $b_{XU,E}$ for AS-SSR systems showed a large variability, indicating that wastewater characteristics, sludge characteristics and operational parameters could influence these rates.

The work presented in this thesis has demonstrated that most sludge reduction can be achieved exposing sludge to aerobic conditions instead of alternating redox conditions. This is important since exposition of sludge to alternating redox conditions was recently described to be key for sludge reduction. Also the strategy to expose sludge to very long SRT, e.g., in current decentralized treatment plants respectively AS-SSR systems, was shown applicable by showing

degradability respectively apparent degradability of endogenous residues. Further research is recommended to show how degradability of endogenous residues is influenced by characteristics of wastewater, sludge and operational conditions. This can improve prediction of sludge reduction and in best-case show applicable process conditions for increased degradation of endogenous residues.

Zusammenfassung

Prozesse, die zur Schlammreduktion (das heisst zur Reduktion der Gesamtschlammasse aus einem System) führen, wurden für kleinere Abwasserreinigungsanlagen typischen Bedingungen untersucht. Kleine Abwasserreinigungsanlagen werden selten betreut, was die separate Schlammbehandlung und -wertschöpfung technisch, ökonomisch und ökologisch schwierig gestaltet. In dieser Doktorarbeit wurden zur prozessintegrierten Schlammreduktion alternierende Redoxpotentiale und hohe Schlammalter angewendet. Das Ziel war, ein besseres Verständnis der Schlammreduktionsmechanismen unter diesen Bedingungen (alternierendes Redoxpotential und lange Schlammaufenthaltszeiten) zu erlangen und die Prozessraten von verschiedenen Schlammreduktionsprozessen zu quantifizieren. Die experimentelle Arbeit bestand aus der Beobachtung der organischen Schlammbestandteile des Belebtschlamms unter verschiedenen Redoxbedingungen und langen Schlammaltern. Zudem wurden diese Beobachtungen mit der Schlammreduktion verglichen, gemessen als Netto-Schlammproduktionskoeffizient (Y_{obs}) oder Abbauraten von organischen Feststoffen. Die folgenden Schlammfraktionen wurden beobachtet: Volatile suspendierte Stoffe (VSS) und deren Bestandteile, die Zellbiomasse (quantifiziert durch intrazelluläres Adenosin tri-Phosphat (ATP)), die extrazellularen polymeren Substanzen (EPS) und die endogenen Reststoffe. Die inerten organischen Feststoffe aus dem Abwasser wurden nur am Rande thematisiert in dieser Doktorarbeit, um den Fokus auf die anderen Schlammfraktionen legen zu können.

Die Prozesse unter alternierenden Redoxpotentialen wurden in Abwasserbehandlungsanlagen vom Typ Belebtschlamm mit Seitenstabilisierung (engl. activated sludge – side stream reactor (AS-SSR)) untersucht. Dieses AS-SSR System besteht aus einem konventionellen Belebtschlammprozess gekoppelt an einen Schlammstabilisierungsreaktor in der Schlammrückführung. Das AS-SSR System wurde bereits in der Forschung zur Untersuchung von Schlammreduktionsprozessen sowie im Grossmassstab zur Abwasserbehandlung mit simultanem Schlammabbau verwendet. Berichte über Systeme im Grossmassstab haben gezeigt, dass die Schlammreduktionsleistung variabel ist und dass diese Variabilität mit dem heutigen Wissensstand nicht genügend abschätzbar ist. Darum ist ein vertieftes Verständnis von Schlammreduktionsprozessen in diesem System relevant für dessen Anwendung. Im Rahmen dieser Doktorarbeit wurde der Seitenstabilisierungsreaktor (SSR) in experimentellen Phasen

unter Fermentationsbedingung (Redoxpotential -380 mV) und spärlich belüfteter Bedingung (Redoxpotential -250 mV) unterschiedlich betrieben, um den Schlamm unterschiedlichen Redoxpotentialen auszusetzen. In einer dritten experimentellen Phase wurde der SSR aerob betrieben. Es hat sich gezeigt, dass sich die organischen Schlammbestandteile der Zellularbiomasse, die EPS sowie die VSS am schnellsten unter belüfteten Bedingungen abbauten (VSS wurde aerob 2.2–4.1-mal schneller abgebaut als mit spärlicher Belüftung). Die grösste Schlammreduktionsleistung unter aeroben Bedingungen war auch die plausible Erklärung für den tiefsten $Y_{obs,}$ der unter aerobem Betrieb des SSRs generell gemessen wurde (Y_{obs} war bis zu 27% tiefer unter aeroben Bedingungen im SSR als unter sauerstoffarmen Bedingungen im SSR). Höhere Schlammalter haben zu tieferen Y_{obs} geführt unter allen drei Betriebsarten des SSRs (Fermentationsbediungung, spärliche Belüftung und aerob). Dies ist wichtig zu erwähnen, weil das Schlammalter oft nicht als Begründung der Schlammreduktionsleistungen in Betracht gezogen wird.

Die Anwendung von sehr hohen Schlammaltern in Abwasserreinigungsanlagen kann nur erfolgreich sein, wenn sich Schlammfraktionen abbauen lassen, die traditionell als inert angeschaut wurden, zum Beispiel die endogenen Reststoffe. Die Abbaubarkeit von endogenen Reststoffen wurde für Belebtschlamm gezeigt, der aus der Behandlung von vorgeklärtem kommunalem Abwasser entstanden ist. Die Abbaurate erster Ordnung für endogene Reststoffe ($b_{XU,E}$) lag bei $0.7–1.1 \cdot 10^{-3}$ d^{-1} unter aerober und unter intermittiert belüfteter Stabilisierung im Batch-Verfahren, bei Raumtemperatur (rund 20°C) während 100 und 380 Tagen. Der Abbau von endogenen Reststoffen wurde damit für realen Belebtschlamm unter Bedingungen nachgewiesen, die in sehr kleinen, dezentralen Anlagen vorgefunden werden können, das heisst bei Raumtemperatur und sehr geringem Kontakt des Schlamms mit aktiver Biomasse. Der Abbauprozess der endogenen Reststoffe war jedoch sehr langsam, annäherungsweise siebenmal langsamer als früheren Veröffentlichungen zufolge ($7 \cdot 10^{-3}$ d^{-1}). Ein scheinbarer Abbau der endogenen Reststoffe wurde auch für die AS-SSR Systeme aufgezeigt, was darauf hinweist, dass die Abschätzung der Überschussschlammproduktion in diesen Systemen verbessert werden kann, wenn der Abbauprozess der endogenen Reststoffe beachtet wird. Die eruierten Abbauraten der endogenen Reststoffe haben eine grosse Variabilität gezeigt. Dies deutet darauf hin, dass Abwasser- und Schlammeigenschaften ebenso wie Betriebsbedingungen die Abbauraten beeinflussen könnten.

Die Untersuchungen in dieser Doktorarbeit haben bewiesen, dass die grösste Schlammreduktion erreicht werden kann, wenn Schlamm aeroben Bedingungen anstelle von alternierenden Redoxpotentialen ausgesetzt wird. Ein alternierendes Redoxpotential wird häufig als Schlüsselaspekt in der Schlammreduktion beschrieben. Auch die Strategie sehr hoher Schlammalter zur Schlammreduktion, zum Beispiel für die gängigen kleinen dezentralen Abwasserreinigungsanlagen und AS-SSR Systeme, hat sich dank dem Nachweis des tatsächlichen Abbaus respektive scheinbaren Abbaus der endogenen Reststoffe als möglich erwiesen. Weiterführende Forschung wird vorgeschlagen, um zu klären, wie die Abbaubarkeit der endogenen Reststoffe durch Abwassereigenschaften, Schlammeigenschaften und Betriebsbedingungen beeinflusst wird oder wie die Abbaubarkeit der endogenen Reststoffe durch gezielte Massnahmen beeinflusst werden kann.

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Chapter 1

General introduction: Biological sludge degradation processes for in-situ sludge reduction

Thesis chapter:
Habermacher, J., Morgenroth, E., Derlon, N.

Background

Wastewater sanitation and sludge management is the basis for human health. On the one hand immediate spread of diseases and toxic compounds is reduced by sanitation and on the other hand wastewater treatment is key for preservation of the environment, what also affects human health indirectly. Wastewater is treated at wastewater treatment plants (WWTPs) of various scales and with diverse technologies. Large and centralized WWTPs can be operated under low specific operational costs and optimized technological conditions due to the economy of scales. However centralized WWT systems require also a wastewater collection system, a sewer. The sewer system in Switzerland generates costs that represent 55% of the total costs for wastewater and sludge treatment (VSA 2011). In order to limit the size and thus the costs for the sewer networks, small centralized and small decentralized treatment systems were implemented in the past also in industrialized countries (Maurer and Herlyn 2006). And since costs for sewer construction are not expected to change very importantly in the future it can be expected that industrialized countries continue to treat part of the wastewater in small centralized or small decentralized systems. In poor regions or locations with very fast population densification small and decentralized WWT systems may be the only option. In poor regions the capital costs for sewer systems might not be bearable and fast local population increase makes planning of centralized infrastructure with long life cycles (WWTP 30 years, sewer 80 years (Maurer and Herlyn 2006)) insufficient. A specific and important aspect of wastewater treatment plants is the handling of sludge. Sludge is composed of solids (organics and inorganics) that come from the influent wastewater and growth of organisms in the activated sludge process. Sludge in the activated sludge process, i.e. secondary sludge, is a resource of active microorganisms that degrade or sorb various soluble pollutants from the waste stream. However sludge collected from the sewage (primary sludge) and the secondary sludge are also hazardous products that are produced in excess from the WWT process. This sludge has to be sanitized and the residue of the sanitation has to be safely disposed. An important aspect in the sludge sanitation is the reduction of sludge mass. Reduction of sludge mass includes degradation of active biomass (potential pathogens), removal of bioavailable substrates, limitation of regrowth of pathogens and reduction of odor production. And the equipment in the downstream sludge handling and disposal process is reduced with sludge reduction in the upstream process. The sludge mass reduction is also important in terms of operational costs. Sludge treatment is listed as being the

most important operational cost factor besides costs for aeration energy by practitioners. Thus the process of sludge mass reduction, in the following only called sludge reduction process, is central to any wastewater treatment system.

The thesis discusses sludge degradation processes in the context of in-situ sludge reduction for activated sludge processes. But there are adjacent research fields that have influenced this research and that could vice-versa be interested in the outcome, such as membrane fouling (Menniti and Morgenroth 2010), membrane bioreactors (Kunzle et al. 2015, Spérandio et al. 2013) and gravity driven membrane filtration for drinking water production (Derlon et al. 2012).

Sludge reduction technologies

Reduction of sludge mass can be classified into physical, thermal, chemical and biological processes or a combination of these processes (Foladori et al. 2010). Physical and thermal processes include disintegration with ultrasound, mills, homogenizers, thermal treatment in the range of 40-180 °C, freezing and thawing (Wei et al. 2003). The chemical processes include ozonation, chlorination, addition of chemical uncouplers, acids, alkali, H_2O_2 (Wei et al. 2003) and hydrolytic enzymes (Foladori et al. 2010, Wawrzynczyk et al. 2008). Though these technologies can reduce sludge they require energy for running the physical and thermal devices or continuous addition of chemicals is needed. The biological sludge reduction methods can present an advantage because the degradation of organic matter is catalyzed by organisms that grow naturally in the sludge or that can once be added and then sustain themselves. For large, centralized WWTPs biological sludge reduction happens frequently in a separate sludge line under anaerobic conditions allowing to recover methane as a source of energy and convert ammonia from the digester supernatant with low energy input by anaerobic ammonium oxidation. At a longer perspective even more valuable goods than methane may be produced from sludge such as building blocks for bioplastic (Johnson et al. 2010, Paul and Liu 2012). But the complete sludge line including anaerobic digestion with biogas utilization requires large installations and regular service to sustain the process and represents a large fraction of the costs of WWTPs. Small treatment systems apply frequently sludge treatment technologies that are different from anaerobic digestion (Svardal and Kroiss 2011). Smaller size, centralized treatment facilities mostly apply simultaneous aerobic stabilization (population equivalent (PE)

< 20’000 (Svardal and Kroiss 2011)) what is an in-situ sludge reduction technology. Other in-situ biological sludge reduction technologies are activated sludge with side-stream reactor for sludge digestion (AS-SSR) (Foladori et al. 2010, Paul and Liu 2012) and systems that are operated at very long SRT such as MBRs (Bengoa et al. 2011, Foladori et al. 2010, Paul and Liu 2012). Small scale, decentralized treatment systems show a diversity of technologies for wastewater treatment, in Switzerland in 2006 an inventory showed the most frequent aerobic systems with PE<500 are trickling filters, activated sludge and infiltration systems (Hieber 2006). These systems can be operated at very long SRT, e.g. with an annual sludge wastage (Hieber 2006). In in-situ sludge reduction technologies the predicted sludge reduction does not always meet expectations, such as in the AS-SSR case (Hering 2012) and this is linked to the none complete understanding of all relevant processes (Johnson et al. 2008). Better understanding of the processes can lead to improved prediction of process performance and eventually to improved process design or operation. The conditions in AS-SSR systems were thus studied and tested for mechanistic explanations. Also sludge reduction at very long SRT has recently gained interests by showing that very long SRTs can lead to degradation of sludge fractions that were traditionally assumed inert, such as endogenous residues and influent unbiodegradable organics (Spérandio et al. 2013). However the degradation kinetics of these sludge fractions remains to be studied under different conditions. This also serves the goal of improved prediction of sludge reduction (at very long SRT) and potentially improved process design or operation.

In-situ biological sludge reduction technologies

The microbial related processes that lead to reduction of sludge mass represent a vast assembly of processes (Hao et al. 2010). The engineering in wastewater treatment uses commonly models of these biological sludge reduction processes. But the assembly of the many real processes is often reduced in numbers, e.g. in ASM (Henze et al. 2000). A brief overview about the processes is given in order to clarify the terminology. Hao et al. (2010) give an overview about endogenous processes in activated sludge. According to them endogenous processes include all processes that lead to reduction of sludge mass. This includes cryptic growth, where microbial cells lyse and the product of the lysis can be partly or fully used as substrate by living cells. If this cell lysate has to be hydrolyzed first, the hydrolysis of cell lysate is also part of the endogenous processes. The cell lysis could be promoted by viruses or predators (bacteriophage,

predatory prokaryotes, protozoans and metazoans (Paul and Liu 2012)). All cell metabolism that is not related to growth is also included such as motility and diverse maintenance functions. This can include oxidation of cell internal material to maintain the critical microbial functions. Utilization of cell internal storage products for growth or maintenance is also included since the net result is always a decrease in mass. The activated sludge models (ASM) from the international water association (IWA) incorporated sludge degradation with the following processes. ASM1 (Henze et al. 2000) includes a death-regeneration concept where active biomass decays and transforms to slowly biodegradable organics (XC_B) and to a smaller fraction into the inert endogenous residues fraction ($X_{U,E}$). This resembles mostly the cryptic growth process. ASM2d (Henze et al. 2000) describes a lysis process, which is similar to the death-regeneration process in ASM1. A novelty of ASM2d in comparison to ASM1 is that specialized heterotrophs are included that carry out phosphorus or glycogen storage (PAOs and GAOs). The storage compounds become bioavailable in the lysis process as well. ASM3 (Henze et al. 2000) includes an endogenous respiration process where active biomass and cell internal storage products are respired with same specific rates. By this, ASM3 biomass degradation concept is closest to the maintenance processes. Though many sludge degradation processes have been observed in microbial studies and modeled in engineering terms, specific applications require a better understanding, especially regarding optimized conditions for low excess sludge production.

Alternating redox conditions and very long solids retention time for biological sludge reduction

Why the understanding of sludge reduction processes is relevant for engineering applications

Sludge reduction by subjecting sludge to alternating redox conditions or to long solids retention time (SRT) are promising technologies to reduce sludge handling, transportation and disposal costs. However, for sludge reduction by alternating redox conditions different mechanisms are used to explain the sludge reduction and there is no general consensus about the validity as the following review shows. But the performance of sludge reduction with alternating redox conditions shows important variations what has led to serious problems for applications such as in the case of a full-scale system using the alternating redox potential (Hering 2012). If the

mechanisms for sludge reduction under alternating redox potentials are understood, the performance prediction for this technology is thought to improve and eventually improved process design is possible. The increase in SRT for sludge reduction has also received attention due to recent findings about the degradability of sludge fractions that were traditionally thought to be inert (endogenous residues ($X_{U,E}$), influent inert organic particulates ($X_{U,Inf}$)). But for the successful application of this long SRT for sludge reduction the degradability of $X_{U,E}$ and $X_{U,Inf}$ has to be demonstrated for some relevant conditions, in some systems the degradation rates require confirmation and in general the dependency of the degradation rate on sludge or process conditions remains at hypothetical level. Also for $X_{U,E}$ and $X_{U,Inf}$, improved knowledge about degradation rates leads to more accurate performance prediction and also improved process design could result. Enhanced sludge reduction (i.e. a faster rate and/or a larger extent of sludge degradation) was reported when sludge was exposed sequentially to different redox conditions (Semblante et al. 2014) or retained for very long time (Spérandio et al. 2013). The possible mechanisms for sludge reduction under these conditions are reviewed and the critical research gaps identified.

Review: Exposure of sludge to alternating redox conditions

Enhanced degradation of sludge that is exposed sequentially to different redox conditions was explained by several mechanisms.

Decay of active microbial cells. Decay of active biomass is rate limiting in active biomass degradation, according to activated sludge models, such as ASM1-2d (Henze et al. 2000). According to these models, if the decay rate can be enhanced, the degradation of active biomass can be enhanced and ultimately sludge degradation could be enhanced. Chen et al. (2003) investigated the decay of active microbial cells under aerobic and oxygen limited conditions. (The oxygen limited condition was achieved by putting activated sludge into a batch reactor for 16 hours without aeration. During the first five hours nitrate was consumed and during the remaining eleven hours the redox potential was at -250 mV). They observed a much faster decrease of active microbial cells under oxygen limited condition than under aerobic conditions. However this was the case only for one out of two activated sludges that were tested. Nevertheless, the TSS decreased very rapidly under oxygen limited condition, for both of these sludges. Therefore TSS degradation under oxygen limited condition was explained by another

mechanisms than enhanced cell decay, at least for one out of these two sludges. The interpretation of Chen et al. (2003) that active microbial cells decay fast under oxygen limited condition and thus lead to a low net sludge yield (Y_{obs}) in a treatment plant can thus not be fully supported. (Part of the inconsistent interpretation in Chen et al. (2003) could come from the application of a method that relies on cell respiration activity, thus a method that is not able to distinguish between inactive and dead cells. However exposure of sludge to 16 hours starvation under oxygen limited conditions could not only lead to cell decay, but also temporary inactivation. However Chen et al. (2003) interpreted all decrease of respiration activity as cell decay). Indeed, Foladori et al. (2015) came to the opposite conclusion that active microbial cells degrade not under anaerobic starvation condition, but they degrade under aerobic starvation condition. From the data in Foladori et al. (2015) it is however unclear how much the decay of active microbial cells caused sludge degradation, because VSS or TSS degradation data was not reported in parallel to the decay measurements.

Degradation of extracellular polymeric substances (EPS). A complementary sludge fraction to the active cellular biomass are the extracellular polymeric substances (EPS). EPS is part of the activated sludge floc matrix and is thus a major constituent of the organic activated sludge mass. It has been demonstrated that EPS is biodegradable (Menniti and Morgenroth 2010) but also that EPS is still part of digested sludge (Park et al. 2008). Therefore, if the rate or extent of EPS degradation can be increased, sludge degradation can be enhanced. Anaerobic solubilization of EPS was described as primary factor for sludge degradation in WWTPs using alternating redox potentials (Chon et al. 2011, Foladori et al. 2015, Novak et al. 2007). However, only two of these studies used indicative measurements for EPS degradation. Novak et al. (2007) showed moderate concentrations of soluble proteins (81 mg L^{-1}) in an anaerobic SSR. However the estimation of EPS solubilization rate in the anaerobic SSR based on the soluble protein measurement is very unprecise, because the concentration of proteins is affected not only by the solubilization process, but also by transformation and transportation processes. From Novak et al. (2007) it can be deduced that some solubilization occurs in an anaerobic SSR, however the intensity of solubilization in comparison to aerobic condition is unknown. Foladori et al. (2015) conducted batch experiments under aerobic and anaerobic starvation conditions with activated sludge. Solubilization of organic matter was observed under anaerobic conditions in absence of cell lysis. Thus EPS solubilization under anaerobic condition

was correctly interpreted by Foladori et al. (2015). Under aerobic condition no accumulation of soluble COD was observed and interpreted as absence of EPS solubilization. However latter interpretation is not supported, because solubilized EPS is to a very large extent biodegradable (Menniti et al. 2009), and will thus be metabolized under aerobic condition rather than accumulate to large extents in the aerated bulk liquid. A direct measurement of EPS degradation under different redox conditions is thus missing. The role of iron has been investigated regarding sludge and especially EPS degradability under anaerobic conditions. Novak et al. (2003) suggested association of organic matter, especially proteins, to iron inside of sludge flocs under aerobic conditions. When sludge is transferred to anaerobic zones iron would be reduced and its associated organic matter be released and become bioavailable. Sludge degradation extent under anaerobic conditions was increased for increased ratios of iron/sodium (Park et al. 2006). Park et al. (2007) showed that EPS extractible with base, thus presumably EPS attached to iron, were more solubilized than other EPS fractions during anaerobic digestion. Following this many AS-SSR studies including an anaerobic SSR referred to this mechanisms as possible cause for low Y_{obs} (Chon et al. 2011, Foladori et al. 2015, Novak et al. 2007) but without showing supporting measurements.

Metabolic uncoupling. The concept of metabolic uncoupling for sludge reduction is explained by Chudoba et al. (1992). This metabolic uncoupling happens when microbial cells are exposed to aerobic feasting and anaerobic starvation conditions according to Chudoba et al. (1992). Chudoba et al. (1992) explained the concept of metabolic uncoupling as followed. Metabolic uncoupling involves on the one hand that under aerobic feasting condition the microbial cells carry out catabolism at a very high rate (catabolism is the "energy producing process"; ordinary heterotrophic organisms oxidize organic substrate and use this process to produce intracellular ATP). The ATP consumption rate during this feasting phase is slower than the ATP production rate. This is because the ATP consuming process is mainly the synthesis of new cellular biomass (anabolism) and this synthesis has a limited maximum rate. The higher ATP production rate as compared to the utilization rate leads to a net accumulation of ATP inside of the cell, in the aerobic feasting phase. The metabolic uncoupling involves on the other hand the depletion of intracellular ATP when the cells are transferred from the aerobic feasting condition to the anaerobic starvation condition. However this depletion of intracellular ATP under anaerobic starvation condition is not associated to growth and therefore associated to energy loss.

Different studies referred to this metabolic uncoupling to explain low excess sludge production in wastewater treatment plants. Chudoba et al. (1992) operated an oxic-settling-anaerobic (OSA) WWTP where the anaerobic sludge holding tank was inserted directly in the sludge recirculation line. A conventional activated sludge (CAS) system with an activated sludge tank, a settler and sludge recirculation (CAS) was operated in parallel as a control system. Chudoba et al. (1992) observed a decrease of ATP pool in the anaerobic sludge holding tank and a lower sludge yield (Y_{obs}) in the OSA system as compared to the CAS system. However to clearly demonstrate metabolic uncoupling a comparable measurement of ATP in an aerobic sludge holding tank would have been required. In addition, the OSA system of Chudoba et al. (1992) had a higher sludge volume and sludge inventory than the control system. The higher sludge amount in the OSA system is another explanation, besides the metabolic uncoupling, for the low Y_{obs} in the OSA system. Saby et al. (2003) operated an OSA system and the results were mechanistic interpreted in Chen et al. (2003). The conclusion was that OSA system does not reduce Y_{obs} by metabolic uncoupling. However, Chen et al. (2003) evaluated the metabolic uncoupling in a batch test under aerobic condition only, but the energy loss in the metabolic uncoupling process is thought to happen under anaerobic condition that follow the aerobic condition (Chudoba et al. 1992). Saby et al. (2003) observed lower Y_{obs} for an OSA system as compared to the Y_{obs} of a control AS system. The decreased Y_{obs} was attributed to low redox potentials in the sludge holding tank. However, also in this case, the sludge amount retained in the OSA system was higher than in the control AS system. With this, also the solids retention time (SRT) was higher in the OSA system as compared to the AS system. And since an increased SRT is known to decrease Y_{obs} (Spérandio et al. 2013), the lower Y_{obs} in the OSA system could also be attributed to the high SRT in that system. A different mechanism of sludge reduction was explained by Huang et al. (2014) which involves however also inefficient utilization of substrate due to cyclic exposition of sludge to different redox conditions. Huang et al. (2014) measured a low excess sludge production (Y_{obs}) of a wastewater treatment plant with an anaerobic side-stream reactor (AS-SSR), in comparison to a conventional AS plant followed by an anaerobic digester. Huang et al. (2014) showed that substrate mineralization was much faster for sludge from the AS-SSR system as compared to sludge from the control system. However it remains undemonstrated that the anaerobic condition in the side-stream reactor is responsible for the inefficient substrate utilization and the low Y_{obs} of the AS-SSR system. An alternative explanation could be, again, the very long SRT.

Transformation of unbiodegradable organics into biodegradable organics. Johnson et al. (2008) proposed a transformation of the endogenous residues ($X_{U,E}$) under anaerobic conditions into particulate, slowly biodegradable organics (XC_B). Once the biodegradable substrate was returned from the anaerobic SSR to the main reactor, it would be metabolized by heterotrophic microorganisms. This mechanism was used to explain very low excess sludge production yields in AS-SSR systems, such as the commercial Cannibal^{TM} system. Since endogenous residues cannot be measured by a standardized analytical method, this mechanism has not been demonstrated directly. The indirect determination of endogenous residues degradation by modeling and simulations is discussed in the following section about very long solids retention time.

Identified research gap and approach to close it. The effect of different redox conditions on sludge reduction is not fully understood. The mechanisms proposed in previous studies missed often comparison between aerobic and anaerobic systems and/or missed to link their mechanistic observations to sludge reduction performance. The effect of alternating redox conditions on sludge reduction performance was often difficult to assess because two important parameters were not constant between the control system and the system with alternating redox potentials. These are the solids retention time that is known to impact on the sludge yield (Spérandio et al. 2013) and the system layout. The SRT was observed in many AS-SSR studies (Chon et al. 2011, Coma et al. 2013, Huang and Goel 2015, Huang et al. 2014, Novak et al. 2007) but often little importance was attributed to SRT for explaining differences in Y_{obs}. The system layout, such as AS-SSR, AS only or AS-Digester was not discussed explicitly in the considered sludge reduction literature. However from classical engineering theory (Gujer 2008) it can be deduced that for example a system with AS-Digester will produce less organic activated sludge than a system with one AS reactor only, if the same overall SRT is applied and also other external conditions (volumetric load, temperature, etc.) are the same. To improve the understanding, the degradation of the sludge fractions of VSS, active biomass and EPS were quantified inside of AS-SSR systems. The active biomass was monitored based on the measurement of intracellular adenosine tri-phosphate (ATP) concentration. ATP was shown to correlate well to intracellular biomass of microbial cells from various environments (Hammes et al. 2010). The assumption was made that the intracellular biomass volume correlates well

with active biomass amount. To assess the impact of different redox conditions on the degradability of the sludge fractions (VSS, active biomass, EPS) the conditions in the SSRs were varied but the overall SRTs and the HRT in the SSRs were kept constant throughout the experiment. Also the degradation process kinetics in the SSRs were compared to the resulting Y_{obs} in Chapter 2 in order to show how relevant the processes in the SSRs are for the Y_{obs}. With this experimental approach the first two mechanisms, decay of active biomass and EPS in the SSR were addressed. The other two mechanisms, metabolic uncoupling and transformation of unbiodegradable organics were not directly addressed. Nevertheless if one of these mechanisms was dominating sludge reduction performance this would have been detected.

Review: Exposure of sludge to very long solids retention time (SRT)

The responsible process for sludge reduction at very long solids retention time (SRT) is usually considered as degradation of very slowly degradable sludge fractions, such as endogenous residues ($X_{U,E}$) and particulate unbiodegradable influent organics ($X_{U,Inf}$). The focus on this process is justified since the slow degradation of activated sludge for up to 150 days has been demonstrated in batch digestions (Jones et al. 2009, Ramdani et al. 2010, Ramdani et al. 2012a). It is even advantageous to accept this process as main explication for sludge reduction at very long SRT because research can focus on development and quantification of a common model (for $X_{U,E}$ and $X_{U,Inf}$ degradation). The acceptance and research on the degradation processes of $X_{U,E}$ and $X_{U,Inf}$ is of course limited to cases where this model is precise enough to improve prediction of sludge reduction performance, what seems to be frequently the case (Menniti et al. 2012, Spérandio et al. 2013). Alternative processes of sludge reduction at long SRT that are less frequently suggested, such as altered storage and lysis behavior (Lobos et al. 2005) will not be addressed in detail. Research on sludge reduction by long SRT can give useful information by determining rates for the degradation of $X_{U,E}$ and $X_{U,Inf}$, identifying factors that could influence these rates and formulation of exact model structures for the decay process of $X_{U,E}$ and $X_{U,Inf}$.

For sludge reduction the role of $X_{U,Inf}$ is thought of similar importance as the role of $X_{U,E}$. The focus of the thesis was chosen on $X_{U,E}$ for the benefit of going into details of this topic. The research on $X_{U,Inf}$ could be facilitated once the decay of $X_{U,E}$ is better characterized.

Endogenous residues degradation rates under relevant conditions for current remote WWTPs. To predict reliably $X_{U,E}$ degradation for small scale remote WWTPs the endogenous residues degradation rate ($b_{XU,E}$) needs to be assessed under relevant conditions, i.e. conditions that could frequently be found in decentralized systems. An inventory of such systems in Switzerland reveals possible characteristics of such systems. The most frequent plants in 2006 in Switzerland were trickling filters, activated sludge plants and filtration systems (Hieber 2006). These systems are operated at ambient temperature, excess sludge may be stored for long time in poorly mixed tanks or solids layers rather than being continuously mixed with active biomass as in an MBR or an AS-SSR. The sludge may reside in the treatment plant for several hundred days and the microbial sludge produced is based on various organic compounds. Thus the sludge degradation conditions in such plants could be close to the conditions from batch digestion at ambient temperature and long duration observation where fresh biomass is however not added. These conditions were studied by Ramdani et al. (2012a) and Martínez-García et al. (2016) however only on sludge that was produced on acetate as sole carbon source. Confirming $X_{U,E}$ degradation under these conditions with sludge produced on real WW is also important because $X_{U,E}$ degradability has been put in doubts in analyses by Ikumi et al. (2014) and Ekama et al. (2007).

Endogenous residues degradation rates for activated sludge – side-stream reactor systems. A WWT system that is operated at very high overall SRTs for sludge reduction is the AS-SSR system where part of the activated sludge is sent to a side-stream digester. The side-stream digester is commonly operated under anaerobic conditions. Only one $b_{XU,E}$ value was reported for an AS-SSR system (0.0235 d^{-1} by Menniti et al. (2012)). Only one available $b_{XU,E}$ value limits the prediction of sludge reduction performance with modeling for the AS-SSR systems. Menniti et al. (2010) have pointed out that for accurate $b_{XU,E}$ estimation in a full-scale AS-SSR plant the influent characteristics and the performance of the trash- and grit removal units need to be accurately modeled. However the approaches for $b_{XU,E}$ estimation by Menniti et al. (2012) used assumptions on the performance of the trash- and grit removal units. Menniti et al. (2012) estimated $b_{XU,E}$ by modeling the relative Y_{obs} decrease from a pre-Cannibal™ plant to a Cannibal™ plant implementation. For AS-SSR systems $b_{XU,E}$ values are important for performance prediction in AS-SSR systems. More values than the provided one ($b_{XU,E}$ = 0.0235 d^{-1}) are required to either get a reliable average value or to get a good range or distribution of

values. In order to get comparable $b_{XU,E}$ values from different AS-SSR plants a uniform and robust approach is required. The uniform approach requires the use of an identical concept (e.g. modeling the absolute Y_{obs} value of an AS-SSR system or modeling the relative Y_{obs} difference between a control AS plant and an AS-SSR system). Further characteristics of the uniform approach need to be the use of an identical model structure and model parameter values. In addition, if the estimated $b_{XU,E}$ also want to be mechanistically meaningful, the model structure and model parameter values need to describe the AS-SSR treatment plant characteristics (e.g. sludge concentration in the AS tank and the SSR) well. Using simplified conditions for AS-SSR systems such as well characterized synthetic wastewater and leaving the trash and grit removal units away decreases the uncertainty of $b_{XU,E}$ estimation in AS-SSR systems. Such simplified systems refer to laboratory systems what brings the additional benefit that conditions to increase or decrease $b_{XU,E}$ can be tested without disturbing the performance of a real WWTP.

Factors influencing the degradation rate of endogenous residues. Prediction of $b_{XU,E}$ can be improved if factors can be identified that influence $b_{XU,E}$. Such factors could be characteristics of the wastewater and sludge or operational conditions. Comparing $b_{XU,E}$ values reported from literature could give hints about influencing factors for $b_{XU,E}$. However the approaches, model structures and model parameter values are individual for most of the studies so that the meaning of such a comparison could be limited. Thus comparison of $b_{XU,E}$ values are more meaningful inside of experimental studies that only used a unique $b_{XU,E}$ estimation approach for $b_{XU,E}$ estimation. Ramdani et al. (2012a) used a similar approach to estimate $b_{XU,E}$ in batch digestion studies and figured out a significant temperature dependency of $X_{U,E}$ (Arrhenius temperature correction factor of 1.033 for intermittent aeration conditions). Further influencing factors on $b_{XU,E}$ that were proposed in literature are shear (Spérandio et al. 2013), pH (Martínez-García et al. 2016) or the oxygen concentration in aerobic dominated systems (Liu and Wang 2015) but these influences remain to be quantified. Further factors that could influence $b_{XU,E}$ could be hypothesized from the factors that led to low Y_{obs} in AS-SSR systems. High influent iron concentrations and short substrate injection times were shown to lead to low Y_{obs} in Yagci et al. (2015). It could be that systems with high influent iron concentration and short substrate injection time lead to higher $b_{XU,E}$. However $b_{XU,E}$ was not estimated from the data of Yagci et al. (2015). An influence of anaerobic conditions and active biomass concentrations on the decay rate of $X_{U,E}$ was proposed in Johnson et al. (2008). The suitability of an $X_{U,E}$ rate expression

including anaerobic conditions and active biomass to model correct Y_{obs} was shown by Menniti et al. (2010) but it was also recognized that a first order rate expression with respect to $X_{U,E}$ would be suitable as well (Menniti et al. 2012). Concerning the influence of active biomass the batch digestion experiment from Ramdani et al. (2012a) showed a $b_{XU,E}$ of 0.0075 d^{-1} what indicates that high $b_{XU,E}$ values can also be achieved in the absence of high amounts of active biomass. For the anaerobic condition as driving force for $X_{U,E}$ degradation (Johnson et al. 2008) it has been indicated that $b_{XU,E}$ is also degradable under predominant aerobic conditions (Spérandio et al. 2013) and that $b_{XU,E}$ is slightly higher under aerobic and intermittent aerated conditions than under anaerobic conditions (Ramdani et al. 2012a). Influencing factors for $b_{XU,E}$ have thus been suggested or could be hypothesized. But most of these influencing factors can not be directly tested because corresponding experimental data is not available, or the corresponding experimental data has not yet been used for $b_{XU,E}$ estimation. Or the reported $b_{XU,E}$ values are of limited comparability because of the different estimation approaches that were applied or because available information on the influencing factors (e.g. active biomass concentration, influent iron concentration, pH, shear, etc.) is not available. Utilization of available experimental data to estimate $b_{XU,E}$ values with a uniform approach holds the potential to learn more about some of the hypothesized influencing factors on $b_{XU,E}$.

Degradability of EPS at very long SRT. There is the possibility to improve the prediction of sludge reduction at very long SRT by making the activated sludge models more detailed. Including EPS explicitly in activated sludge models could be a reasonable step considering activated sludge flocs contain large amounts of bound EPS, up to 40% (Ramdani et al. 2012b). EPS was not explicitly modeled in commons models such as ASM1-3 (Henze et al. 2000) and also recent modeling efforts on sludge reduction processes considered the two traditional compounds of active biomass and endogenous residues only (Labelle et al. 2015, Martínez-García et al. 2016, Ramdani et al. 2010, Ramdani et al. 2012a, Spérandio et al. 2013). In traditional activated sludge models EPS are considered implicitly within the activated sludge organics. (This is because the model parameters of the traditional models are determined based on COD, VSS, TSS or O.U.R. measurements typically. And these measurements are always influenced by the EPS that is present in the activated sludge). Explicit modeling of EPS was discussed in Laspidou and Rittmann (2002b) and used for instance to model putative membrane foulants (Menniti and Morgenroth 2010). For sludge reduction at very long SRT it would be

meaningful to include EPS explicit into modeling in case implicit modeling of EPS was not precise. This means explicit EPS modeling could be interesting if the EPS can not be described as being a fixed fraction of active biomass and being a fixed fraction of endogenous residues. The question whether EPS can be described as being a fixed fraction of active biomass and endogenous residues can be answered by measuring EPS dynamic in batch digestion and compare it to the dynamic of active biomass and endogenous residues. High degradation rates for overall bound EPS (b_{EPS}, in a first order degradation model) were measured for activated sludges that contain a fair amount of active biomass (less than two days). Degradation rate (b_{EPS}) of around 0.12 d^{-1} (Menniti and Morgenroth 2010) and 0.17 d^{-1} (Laspidou and Rittmann 2002a) were reported. These values of b_{EPS} are in the same order of magnitude than the decay rates of heterotrophic biomass e.g., 0.24 d^{-1} (Dold 2007). Bound EPS thus seem fairly biodegradable during the first days of digestion of activated sludge. Lower values of b_{EPS} were observed during longer periods of digestion (21 days), b_{EPS} was calculated 0.06-0.07 d^{-1} for two activated sludges based on the experimental data reported by Ramdani et al. (2012b). The high b_{EPS} at the initial stage of sludge degradation and the lower degradability at the later stage of digestion indicate that part of the EPS is associated to active biomass and degrades fast and another part of EPS is associated to endogenous residues and thus does not degrade or degrade only slowly. But at very long SRTs (> 30 days in batch digestion) it is unknown whether the degradation of EPS is proportional to the decay of endogenous residues. In addition, the experimental studies that showed EPS dynamics used only one specific sludge (Menniti and Morgenroth 2010, Ramdani et al. 2012b), thus differences in EPS degradability as function of sludge has not been thoroughly addressed. From a mechanistic point of view the different kinetics of EPS degradation (initial high b_{EPS} then low b_{EPS}) could be caused by a heterogeneous arrangement of EPS within activated sludge flocs (Ramdani et al. 2012b).

Sludge and effluent quality. Long SRT could lead to operational problems linked to deteriorated sludge quality and deteriorated effluent quality. The question of sludge and effluent quality is thus important for long SRT systems. Soluble COD in the effluent of long SRT activated sludge systems and systems with sludge reduction by ozonation were reported (Boehler and Siegrist 2006). Other reported problems at very high SRTs (60-80 days) were high effluent suspended solids concentrations and high sludge volume index by Chon et al. (2011). But Chon et al. (2011) also showed that a WWTP consisting of one activated sludge tank only

had more problems (i.e. higher SVI and higher effluent TSS) than a system with AS-SSR layout. Thus sludge and effluent quality in different high SRT systems require clarification.

Identified research gap and approach to close it. The degradability of endogenous residues under conditions that are relevant for common remote WWTP-types was shown for sludge produced on artificial WW only. For successful application of very long SRTs at these remote WWTPs confirmation of $X_{U,E}$ degradation is required for sludge produced on real WW. The degradability of sludge grown on filtered primary influent was assessed in batch digestion studies at ambient temperature for a duration of 380 days without addition of fresh biomass. For another type of WWTP that can be used for sludge reduction, i.e. the AS-SSR system, the sludge reduction performance is subject to important uncertainty because $b_{XU,E}$ values are scarce and of limited comparability due to different approaches that were applied for $b_{XU,E}$ estimation. Values of $b_{XU,E}$ from reported AS-SSR systems with well characterized influents were estimated with a uniform and robust approach in order to deliver comparable values. The estimated $b_{XU,E}$ values were also compared to factors that could influence $b_{XU,E}$, these factors were overall SRT, influent wastewater iron concentration and substrate injection duration. Considering floc bound extracellular polymeric substances as a separate state variable in models could eventually improve sludge reduction prediction. But such an improvement is mainly expected if EPS cannot be well described as being part of active biomass and being part of endogenous residues. The EPS data from literature could be well described with the prior concept (bound EPS is partly attributed to active biomass and partly attributed to endogenous residues) for the first 21 days. This was shown for a single sludge produced on artificial wastewater. In this thesis EPS dynamic from three different sludges was measured for a digestion period of 260 days and compared to dynamic of active biomass and endogenous residues.

Sludge reduction technologies that are implemented in the main process of wastewater treatment may not be of practical relevance if sludge and effluent quality are deteriorated strongly. Reports show that sludge reduction technologies are associated to the risk of producing low sludge and effluent quality but that some systems may be less sensitive to this. In the conducted AS-SSR experiments in this thesis sludge and effluent quality was investigated.

Summary of research questions

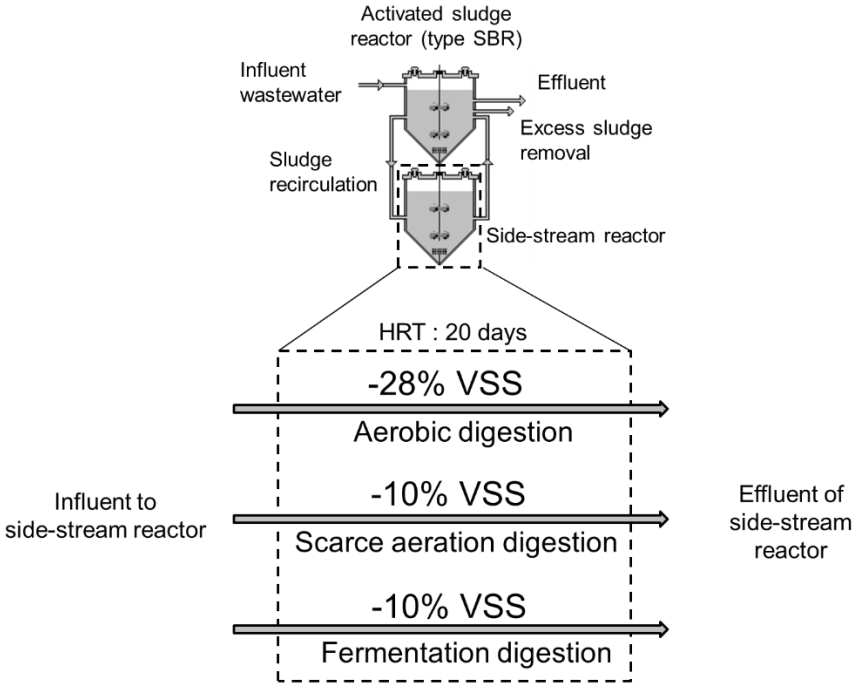
- How is the degradation rate of different sludge fractions (VSS, active biomass, EPS) affected when sludge is exposed to different redox conditions sequentially in activated sludge – side-stream reactor (AS-SSR) systems?
- How do the processes under the different redox conditions affect the sludge reduction performance (Y_{obs}) of a treatment plant that applies these different redox conditions to the sludge in the side-stream digester?
- What is the effect of overall SRT on the sludge reduction performance of AS-SSR systems?
- What is the effluent and the sludge quality of AS-SSR systems?
- Are endogenous residues from waste activated sludge, produced on municipal primary effluent, degradable under conditions that can be found in current small scale, decentralized wastewater treatment systems? Typical conditions that can be found are ambient temperature and low presence of active biomass surrounding the endogenous residues.
- What is the degradability of EPS at very long degradation time? Does EPS stay a part of endogenous residues or does it show a clearly higher or lower degradability than endogenous residues?
- Is there an apparent degradation of endogenous residues in wastewater treatment systems of type activated sludge – side-stream reactor where solids retention time reach high SRTs (30 days) to very high SRTs (more than 100 days)? How sensitive is the estimation of endogenous residues apparent degradation rates towards the models that are used for their estimation? Are there factors that influence endogenous residues decay such as influent wastewater composition or operational conditions?

Chapter 2

Exposure of activated sludge to different redox conditions for sludge reduction

Habermacher, J., Benetti, A.D., Derlon, N., Morgenroth, E., 2015. The effect of different aeration conditions in activated sludge – Side-stream system on sludge production, sludge degradation rates, active biomass and extracellular polymeric substances. Water Research 85, 46-56. DOI: 10.1016/j.watres.2015.08.002

Graphical abstract



Highlights

- Long SRT was shown to decrease excess sludge production
- Organic solids (VSS), active biomass and EPS degraded most under aerobic condition
- EPS hydrolyzed simultaneously to VSS
- Long SRT did not deteriorate effluent quality of the activated sludge process

Abstract

On-site minimization of excess sludge production is a relevant strategy for the operation of small-scale and decentralized wastewater treatment plants. In the study, we evaluated the potential of activated sludge systems equipped with side-stream reactors (SSRs). This study especially focused on how the sequential exposure of sludge to different aeration conditions in the side-stream reactors influences the overall degradation of sludge and of its specific fractions (active biomass, extracellular polymeric substances (EPS), EPS proteins, EPS carbohydrates). We found that increasing the solid retention time from 25 to 40 and 80 days enhanced sludge degradation for all aeration conditions tested in the side-stream reactor. Also, the highest specific degradation rate and in turn the lowest sludge production were achieved when maintaining aerobic conditions in the side-stream reactors. The different sludge fractions in terms of active biomass (quantified based on adenosine tri-phosphate (ATP) measurements), EPS proteins and EPS carbohydrates were quantified before and after passage through the SSR. The relative amounts of active biomass and EPS to volatile suspended solids (VSS) did not changed when exposed to different aeration conditions in the SSRs, which indicates that long SRT and starvation in the SSRs did not promote the degradation of a specific sludge fraction. Overall, our study helps to better understand mechanisms of enhanced sludge degradation in systems operated at long SRTs.

Keywords

side-stream reactor, endogenous decay, starvation, solid retention time, EPS, exoenzymes.

Nomenclature

$b_{X,SBR}$	specific sludge degradation rate of the SBR (d^{-1})	$S_{NO3\text{-}N,effl}$	nitrate-nitrogen concentration in the effluent (mg NO_3-N L^{-1})
$b_{X,SSR}$	specific sludge degradation rate of the SSR (d^{-1})	$S_{N2\text{-}N,effl}$	nitrogen gas – nitrogen concentration in the effluent (mg N_2-N L^{-1})
$\Delta (X_{SBR} * V_{SBR}) \Delta t^{-1}$	solids accumulation rate in the SBR measured over one or two weeks period (mg VSS d^{-1})	V_{SBR}	volume of the SBR (L)
$\Delta (X_{SSR} * V_{SSR}) \Delta t^{-1}$	solids accumulation rate in the SSR measured over one or two weeks period (mg VSS d^{-1})	V_{SSR}	volume of the SSR (L)
$f_{CV,X,Bio}$	conversion factor COD to VSS for active biomass (mg COD mg VSS^{-1})	X_{effl}	solids concentration in the effluent (mg VSS L^{-1})
Q_{effl}	effluent flow rate (L d^{-1})	X_{ex}	solids concentration in excess sludge flow (mg VSS L^{-1})
Q_{ex}	excess sludge flow rate (L d^{-1})	X_{SBR}	solids concentration in the SBR (mg VSS L^{-1})
Q_{in}	influent wastewater flow rate (L d^{-1})	X_{SSR}	solids concentration in the SSR (mg VSS L^{-1})
$Q_{interchange}$	Interchange flow rate (L d^{-1})	$X_{SBR \rightarrow SSR}$	solids concentration in the interchange flow towards the SSR (mg VSS L^{-1})
$S_{COD,effl}$	soluble COD concentration in the effluent (mg COD L^{-1})	$X_{SSR \rightarrow SBR}$	solids concentration in the interchange flow towards the SBR (mg VSS L^{-1})
$S_{COD,in}$	soluble COD concentration in the influent (mg COD L^{-1})	Y_{ANO}	growth yield of autotrophic nitrifying organisms per produced NO_3-N (mg COD mg NO_3-N^{-1} or mg COD mg N_2-N^{-1})
$S_{NO3\text{-}N,effl}$	nitrate-nitrogen concentration in the effluent (mg NO_3-N L^{-1})	Y_{OHO}	growth yield of heterotrophs (mg COD mg COD^{-1})

Introduction

Future wastewater management will be characterized by a greater diversity of wastewater streams that will be treated separately and at different scales (household scale, neighbourhood scale, large centralized scale). Selecting the appropriate technology for the treatment of wastewaters and sludge depends on the scale of the facility (Svardal and Kroiss, 2011). Therefore, a larger diversity of treatment approaches is needed. Sludge treatment in large wastewater treatment plants will combine multiple unit processes and anaerobic digestion for energy recovery while for small treatment plants a simpler approach with reduced capital and maintenance costs will be needed. The research and development of adapted technologies for small scale treatment facilities is on-going (Foladori et al., 2010). A relevant approach for maximizing the decay and hydrolysis of sludge consists in sequentially exposing the sludge to starvation conditions in a side-stream reactor (SSR) placed on the sludge return loop. Such a system is known as activated sludge – side-stream reactor (AS-SSR). Commonly the SSR is operated under anoxic or anaerobic conditions and helps to significantly reduce the observed sludge yield (Y_{obs}) (Semblante et al., 2014).

At laboratory-scale, diminutions of 50% to 60% of the Y_{obs} were reported for AS-SSR systems compared to conventional AS systems (Chon et al., 2011; Huang et al., 2014; Novak et al., 2007). Different operating conditions applied in the SSRs were tested and helped to reduce the Y_{obs}: increased sludge recirculation rate between the main reactor and the SSR (Novak et al., 2007), increased frequency of the sludge recirculation (Sun et al., 2010), fast filling of the main reactor (Yagci et al., 2015), and anaerobic conditions in the SSR (Chon et al., 2011). Especially, maintaining anoxic or anaerobic conditions in SSRs has been reported to be a key aspect for maximizing the sludge degradation (Chon et al., 2011; Coma et al., 2013; Johnson et al., 2008; Novak et al., 2007; Semblante et al., 2014). But the exact mechanisms of enhanced sludge degradation under anoxic or anaerobic conditions in SSRs were not clearly identified and a multitude of different processes were thus proposed to explain the experimental observations. The lack of clear understanding of mechanisms of enhanced sludge degradation in AS-SSR leads to investigations in full-scale systems (Johnson et al., 2008). Inefficient applications of AS-SSRs may be explained by our limited understanding of the mechanisms responsible for the enhanced biomass decay and hydrolysis, especially within the SSR.

The solid retention time (SRT) represents on the other hand a key operational parameter for net sludge production in activated sludge systems (Sperandio et al., 2013). Increasing the SRT decreases the Y_{obs}. In previous studies on AS-SSR systems, the SRT was however not always controlled and increased as a result of the addition of a side-stream reactor. It is thus not possible to clearly identify if the reduced Y_{obs} observed in AS-SSR systems results from the increased SRT or from the specific aeration conditions maintained in the SSRs. Only Chon et al. (2011) investigated the effect of the aeration condition independently of the system configuration and SRT. In their study, Chon et al. (2011) observed similar Y_{obs} for aerobic or anaerobic conditions maintained in the SSRs. Their observations suggest that the reduced Y_{obs} observed for AS-SSR systems could result from the increased SRT and not from specific degradation mechanisms occurring in the SSRs. Also, enhanced sludge degradation observed under anoxic or anaerobic conditions contradicts several experimental observations that reported increased sludge degradation rates under aerobic conditions (Gujer et al., 1999; Siegrist et al., 1999). A first objective of the present study thus aims at clarifying previous observations of enhanced sludge degradation under alternating redox conditions.

Concerning the mechanisms of sludge reduction in AS-SSR systems, previous studies suggested that the sequential exposure of sludge to starvation conditions in the SSR increased the biodegradability of individual sludge fractions (extracellular polymeric substances, endogenous residues) (Johnson et al., 2008; Novak et al., 2007). In experiments where sludge was exposed sequentially to anaerobic or intermittent aeration conditions the sludge reduction was explained by different sludge pools, which are only degradable under specific aeration conditions (Chon et al., 2011; Novak et al., 2007; Ramdani et al., 2012). This explanation is based on batch digestion studies with waste activated sludge by Park et al. (2006). In their study, different cations and residual biopolymers were found in solution after digestion, in function of the digesting conditions (aerobic vs. anaerobic). Complementary processes in the anaerobic SSR were also suggested in Johnson et al. (2008). However, an accurate characterisation of the sludge composition and quantification of the sludge degradation that occurs in the SSR are still missing. A second objective of the present study is to better understand if specific sludge fractions are degraded in the SSR, and if specific aeration conditions help to enhance their biodegradability.

The following questions were thus addressed in this study (i) what is the specific effect of SRT and aeration conditions in the SSR on the Y_{obs}? (ii) Are specific processes taking place in the SSRs such as preferential degradation of active biomass, degradation of extracellular polymeric substances (EPS), or enhanced enzymatic hydrolysis? To address these questions, three systems treating synthetic wastewater were operated at different SRTs of 25, 40 and 80 days. Each system consisted of an activated sludge reactor coupled to a side-stream reactor. Different aeration conditions were tested in the side-stream reactor. The solid degradation was evaluated quantitatively through the measurement of the Y_{obs} and the specific sludge degradation rates. Sludge composition was characterised in terms of active biomass fraction (through ATP measurements), EPS proteins and EPS carbohydrates and hydrolytic exoenzymatic activities.

Materials and methods

Experimental set-up and operation

The experimental systems consisted of two reactors: a sequencing batch reactor (SBR) connected to a side-stream reactor (SSR) (Fig. 2.1). The AS-SSR sequence was as follow: filling of the SBR with fresh synthetic wastewater (10 min) under anoxic conditions, aerobic reaction phase in SBR (287 min), excess sludge removal from the SBR (6 min), settling in SBR (40 min), supernatant removal from the SBR (10 min), recycling of sludge between SBR and SSR (7 min) while both reactors were mixed. The recirculated sludge volume was 0.125 L per cycle, which equals 0.5 L d^{-1}. In the SSRs the sludge was digested under different aeration conditions depending on the experimental phase. The SSRs were continuously stirred using a mechanical mixer. Online measurements of O_2, temperature, redox potential, pH were performed. Temperature was controlled at 20°C using a thermostat. Alkalinity was provided in excess with the synthetic wastewater to stabilize pH. In the last of the experimental phases (see below) the pH in the SSRs was also controlled by HCl injection.

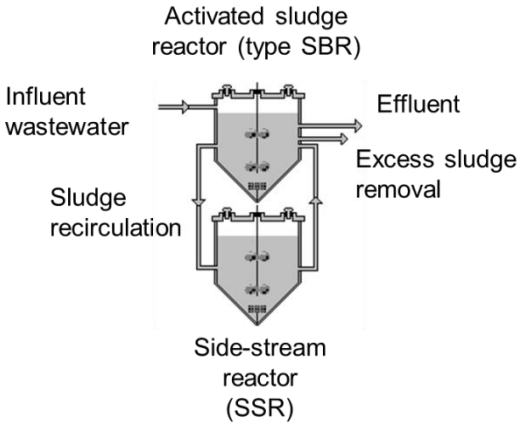


Fig. 2.1 Schematic representation of one experimental system including the sequencing batch reactor (SBR) and the side-stream reactor (SSR).

Experimental approach

The three AS-SSR systems were operated in parallel at different overall SRTs: system "25 d SRT", system "40 d SRT" and system "80 d SRT". The calculation of the overall SRT is described in the following section *calculations*. Systems were operated at different SRTs by maintaining (i) different flow-rates of excess sludge removal and (ii) different mixed liquor volumes in the SSRs (Table 2.1). Three experimental phases were conducted. In the first phase (15 months), the SSRs were operated under fully aerobic conditions (oxygen concentration was kept at 2 - 6 mg O_2 L^{-1}) controlled by on-off aeration. In a second phase (5 months), a redox potential of -250 mV was maintained in the SSRs through on-off aeration, inspired by the experiments of Saby et al. (2003). In this scarce aerated phase, the oxygen concentration remained below the detection limit of 0.001 mg O_2 L^{-1} during more than 90% of the operation time. Only after the short aeration intervals, latter lasting for 10 to 30 seconds, the oxygen concentration overpassed occasionally the detection limit to reach values up to 0.11 mg O_2 L^{-1}. Concentrations of nitrite and nitrate, measured bi-monthly, were below 1 mg N L^{-1}. In a third phase (3 months) the SSRs were operated with daily aeration period of 1 hr (23 hrs non aerated). During this 1 hr aeration period the oxygen concentration was maintained between 0.2 and 1 mg O_2 L^{-1} to allow for fermentation of activated sludge but inhibition of the methanogenesis

(Ramdani et al. (2012)). The redox potential value was stable at -380 mV in the non-aerated period, and reached about -100 to +100 mV during the 1 hr aeration period. These different experimental phases are named "SSRs aerobic", "SSRs scarce aeration" and "SSRs fermentation" in the following in this article.

Table 2.1 : Characteristics of the systems and reactors (SRT = solid retention time, HRT = hydraulic retention time, SBR = sequencing batch reactor, SSR = side-stream reactor).

		Nominal overall solid retention time (SRT) and type of reactor (SBR or SSR)					
		"25 d SRT"		"40 d SRT"		"80 d SRT"	
Parameter	Experimental phase	SBR	SSR	SBR	SSR	SBR	SSR
Reactor volume (L)	All phases	10	6	10	8	10	10
HRT (d)	All phases	0.625	12	0.625	16	0.625	20
Mixed liquor removed from SBR to control SRT [a], average values (L d^{-1})	SSRs aerobic	0.67	-	0.44	-	0.21	-
	SSRs scarce aeration	0.68	-	0.56	-	0.31	-
	SSRs fermentation	0.76	-	0.48	-	0.25	-

[a] Mixed liquor removal was adjusted in response to systems performance to operate the system close to the nominal overall SRTs.

Synthetic wastewater preparation

Synthetic wastewater (WW) was used in this study. Nutrients such as N and P were provided in excess (COD/N/P ratio of 100/15/3) (Table 2.2). The influent COD was in average 550 mg COD L^{-1} and was composed of different carbon sources (Table 2.2). The stock solution containing the nutrients was prepared weekly and stored at 4 °C. The stock solution was then diluted with tap water during the filling period.

Table 2.2 : Recipe of the synthetic wastewater (two columns to the left) and compounds measured in the influent (two columns to the right)

Added compound	Design influent concentration (mg L^{-1})	Measured compound	Measured influent concentration (mg L^{-1})
Sodium-Acetate	196	Na^{+}	305
Peptone	196	Cl^{-}	166
Yeast extract, (hydrolyzed)	196	Ca^{2+}	101
NH_4Cl	174	K^{+}	52.5
KH_2PO_4	37	SO_4^{-}	23
K_2HPO_4	59	Mg^{2+}	16
$NaHCO_3$	1054	Iron	(b.d.)[a]

[a] b.d. (detection limit) = 0.05 mg L^{-1}

Analytical methods

Sludge sampling, suspended solids, chemical parameters

Sludge from the SBRs and SSRs was sampled weekly or biweekly in duplicate for VSS and TSS measurements according standard methods (APHA et al., 2005). Measurements of sludge volume index (SVI), dewaterability and COD were measured according to standard methods (APHA et al., 2005). Dewaterability measurements were performed according the time-to-filter method (TTF) with 100 ml sludge. Soluble nitrogen compounds, phosphate and divalent cations were analysed by ion chromatography (IC) (Metrohm, 761 Compact IC) after filtration at 0.45 µm using glass-fiber filters.

ATP measurements

Total and soluble ATP concentrations were measured in the sludge of the SBRs and SSRs using BacTiter-Glo (Promega Corporation) kit and protocol. The soluble ATP was measured on sludge filtrate (0.45 µm glass-fiber filters) and was sampled simultaneously to sludge samples for total ATP determination. Sludge from the SBRs was sampled at the end of the reaction phase, at the same time sludge from SSRs was sampled. All sludge samples were diluted 10 to 50 times in deionized water before measurement. 100 µl of sample was mixed with 100 µl of reagent. The reaction was carried out at 38 °C with 10 seconds incubation time on vortex. Triplicates samples followed by triplicates analyses on each sample were performed yielding nine measurement values per reactor and sampling date. The intracellular ATP concentrations were calculated as the difference between total and soluble ATP. The intracellular ATP concentration was normalized by the VSS concentration of the sludge that was used for ATP-assay.

EPS extraction and quantification

EPS extraction and analyses were carried out according to a multi-extraction protocol based on sonication, Tween and EDTA (Ras et al., 2008). The following points were modified from the original protocol proposed by Ras et al. (2008): sludge concentrations were adjusted to 7 g VSS L^{-1} before the extraction. For protein quantification from EDTA extracts 10 µl (instead of 50 µl) of sample were added to 400 µl reagent. For the carbohydrates determination, all volumes

from the original protocol were divided by two. All extracts were analysed in duplicates for proteins and carbohydrates. Similar to Ras et al. (2008), cell lysis was controlled using the protocol based on G6P-DH activity measurements (Lessie and Wyk, 1972). No cell lysis was detected in any of the extracts except in the positive control samples containing purified G6P-DH. The measured EPS concentrations were normalized by the initial VSS of the sludge that was used for EPS extraction, i.e., 7 g VSS L^{-1}.

Enzymatic activities

Different substrates that undergo microbial, extracellular hydrolysis were used to determine hydrolytic enzymatic activities of mixed liquor or of the supernatant (Table 2.3). The standard protocol for end-point measurements proposed by Kemp (1993) was applied with the following specifications. Samples were taken at the end of the SBR-reaction phase and diluted 5 times in Tris-HCl solution (final Tris concentration 10 mM, final pH 7.5). Enzyme substrates were then added to reach a final concentration of 1 mM, reaching a reaction volume of 1 ml. Incubation was performed for 10 or 20 minutes under continuously mixed conditions. The reaction was stopped by adding 1 ml of glycine buffer (1 M Glycine, 4 M NH_4OH in deionized water, pH 10.3). Samples were then centrifuged, the supernatant was collected and fluorescence was measured. The fluorescence of the protease assay was determined at excitation/emission wavelengths of 340/436 nm. For all the other assays excitation/emission wavelengths of 366/442 nm were used. Obtained fluorescence was translated into concentration of fluorophore molecules by standard curves that were produced with the pure fluorophores. All assays were carried out in triplicates at ambient temperature (19-24 °C). Enzymatic activity measurements were always performed simultaneously for all reactors. One negative control measurement was carried out per assay and signals from samples were corrected for that blank signal. Since the assays were carried out at controlled pH and specific temperatures, the hydrolytic activity measurements indicate the abundance of the hydrolytic exoenzymes in the sludge samples rather than the activity inside of the reactors (which is dependent on pH and temperature). The measured enzymatic activities were normalized by the VSS of the sludge that was used for enzymatic activity assays and the duration of the assay.

Table 2.3 : Enzymes targeted in the assays and corresponding substrates.

High classification of enzyme	Name of enzyme	EC-Number of enzyme (BRENDA, 2007)	Substrate used to target the enzyme	Sigma-Aldrich prod. number
EC 3.4 Peptidases	leucyl aminopeptidase	3.4.11.1	L-Alanine 7-amido-4-methylcoumarin trifluoroacetate salt	A4302
EC 3.1.3 Phosphoric Monoester Hydrolases	Alkaline phosphatase	3.1.3.1	4-Methylumbelliferyl phosphate disodium salt	M8168
EC 3.2.1 Glycosidases	β-N-acetylhexosaminidase	3.2.1.52	4-Methylumbelliferyl N-acetyl-β-D-glucosaminide	M2133
EC 3.2.1 Glycosidases	α-galactosidase	3.2.1.22	4-Methylumbelliferyl α-D-galactopyranoside	M7633
EC 3.2.1 Glycosidases	α-glucosidase	3.2.1.20	4-Methylumbelliferyl α-D-glucopyranoside	M9766
EC 3.2.1 Glycosidases	β-galactosidase	3.2.1.23	4-Methylumbelliferyl β-D-galactopyranoside	M1633
EC 3.2.1 Glycosidases	β-glucosidase	3.2.1.21	4-Methylumbelliferyl β-D-glucopyranoside	M3633

Calculations

The overall SRT was calculated as the total mass of VSS in the system (SBR + SSR) divided by the daily VSS mass flux that was removed from the system. The HRT of the SSR was defined as the working volume of the SSR divided by the daily liquor flow through the reactor, idem for the SBR. The observed sludge yield, Y_{obs}, was calculated as the net production rate of VSS mass (mg VSS d^{-1}) over the rate of substrate-COD elimination from the wastewater (mg COD d^{-1}) (Tchobanoglous et al., 2003). Sludge removal from the systems due to effluent solids, sampling and reactor cleaning were considered in the calculations of SRT and Y_{obs} (and also in the calculations of specific sludge degradation rates b_X, see calculations below). Individual SRTs and Y_{obs} were calculated for all periods between two consecutive VSS sampling dates, excluding periods with operational incidents. The average for the three different experimental periods was calculated subsequently and represented with the standard error estimate. The same applies for b_X calculations (see following).

The specific sludge degradation rates (b_X) were calculated using eq. (2.1) for SSRs and eq. (2.2) for SBRs (Fig. 2.2 illustrates the variables, a detailed development of the equations is found in the SI, symbols used in the equations are described in the Nomenclature).

$$b_{X,SSR} = \left(Q_{interchange} * (X_{SBR \rightarrow SSR} - X_{SSR \rightarrow SBR}) - \frac{\Delta(X_{SSR} * V_{SSR})}{\Delta t}\right) * \frac{1}{X_{SSR} * V_{SSR}} \quad (2.1)$$

$$b_{X,SBR} = \left(Q_{in} * (S_{COD,in} - S_{COD,effl}) * Y_{OHO} * \frac{1}{f_{CV,X,Bio}} + Q_{in} * (S_{NO3-N,effl} + S_{N2-N,effl}) * Y_{ANO} * \frac{1}{f_{CV,X,Bio}} - b_{X,SSR} * X_{SSR} * V_{SSR} - (Q_{ex} * X_{ex} + Q_{effl} * X_{effl}) - \left(\frac{\Delta(X_{SBR} * V_{SBR})}{\Delta t} + \frac{\Delta(X_{SSR} * V_{SSR})}{\Delta t}\right)\right) * \frac{1}{X_{SBR} * V_{SBR}} \quad (2.2)$$

Parameters of eq. (2.1) and eq. (2.2) were measured variables from our experiment, unless specified below. The nitrogen gas effluent concentration, $S_{N2—N,effl}$, were deduced from the disappearance of NO_3-N during the anoxic filling phase, which was measured for each SBR individually. Standard microbial growth yields were considered for heterotrophs (Y_{OHO} = 0.67 mg COD mg COD^{-1}) and autotrophs (Y_{ANO} = 0.24 mg COD mg NO_3-N^{-1} or mg COD mg N_2-N^{-1}) (Henze et al., 2000). A conversion factor $f_{CV,X,Bio}$ of 1.42 mg COD mg VSS^{-1} was considered (Sperandio et al., 2013).

Sensitivity of Y_{obs} towards $b_{X,SSR}$ was determined based on an analytical steady-state expression. The purpose was to evaluate whether the experimentally determined relation of Y_{obs} and $b_{X,SSR}$ could be described by a simplified model equation. The approach is described in the supplementary information.

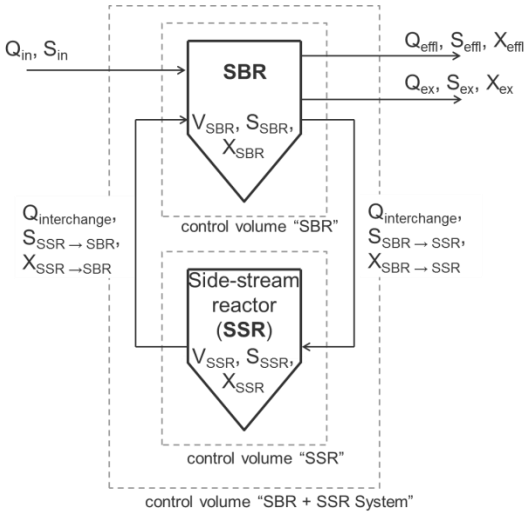


Fig. 2.2 Scheme of a sequencing batch reactor (SBR) and its side-stream reactor (SSR). Concentrations for soluble (S) and particulate (X) compounds, volumes (V), and flow rates (Q) and three control volumes are indicated.

Results

Influence of aeration conditions and solid retention time on observed sludge yield

The observed sludge yields (Y_{obs}) measured during the different experimental phases are plotted as a function of the overall SRT (Fig. 2.3). The lowest Y_{obs} were observed under fully aerobic conditions in the SSRs (black dots, Fig. 2.3). For the systems with overall SRT of 40 and 80 d, the sludge yields were 14 to 27% lower with aerobic conditions in the SSRs as compared to scarce aeration and fermentation conditions. Increasing the overall SRT also significantly decreased the Y_{obs}, especially under fully aerobic and fermentation conditions in the SSRs. For example, increasing the SRTs from approximately 25 to 80 d helped to reduce the Y_{obs} by 13 - 36% independently of the aeration conditions of the SSRs. Under scarce aeration in the SSRs,

the reduction of the Y_{obs} with an increasing SRT was noticeable only when the SRT was controlled at very high values i.e., around 80 d.

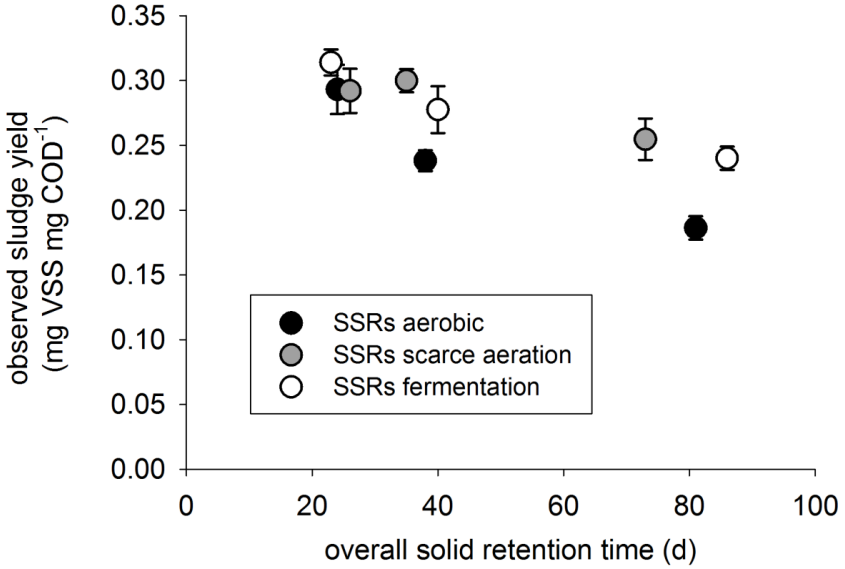


Fig. 2.3 Observed sludge yields (Y_{obs}) measured for the AS-SSR systems operated at different solid retention times and different aeration conditions in the SSRs. Average values of the experimental periods and corresponding standard errors are displayed.

Influence aeration conditions on specific sludge degradation rates

The specific sludge degradation rates (b_X) were calculated for the SBRs and SSRs (Fig. 2.4). Maintaining aerobic conditions in the SSRs increased significantly the specific sludge degradation rates calculated for the SSRs (Fig. 2.4, grey bars). The specific sludge degradation rates were 2.2, 2.7, and 4.1 times higher under aerobic conditions as compared to scarce aeration of the SSRs at SRTs of 25, 40 and 80 d, respectively (Fig. 2.4 (a) and (b), grey bars). A similar trend was noticed when comparing the specific sludge degradation rates of the SSRs for the aerobic and fermentation conditions (Fig. 2.4 (a) and (c), grey bars). In turn, a change in the aeration conditions in the SSRs had only little effect on the specific sludge degradation rates

calculated for the main reactors (SBRs) (Fig. 2.4, black bars). Significant sludge degradation occurred in both the SBRs and the SSRs (Table S 2.1 - SI). Under aerobic conditions, 43 to 49% of the overall sludge degradation occurred in the SSRs. Under the scarce aerated and fermentation conditions, only 21 to 44% of the overall sludge degradation occurred in the SSRs. The SSRs contained on the other hand 39-59% of the overall sludge mass. A sensitivity analysis of Y_{obs} towards $b_{X,SSR}$ was performed. It indicates that at the lowest SRT of 25 days a significant variation of $b_{X,SSR}$ (50%) has a limited influence on the Y_{obs} prediction (14%). On the contrary, the influence of $b_{X,SSR}$ on Y_{obs} is more pronounced at high SRT. For the system with the highest SRT (80 d), Y_{obs} is expected to increase by more than 35% ($b_{X,SSR}$ decreased by ca. 75%).

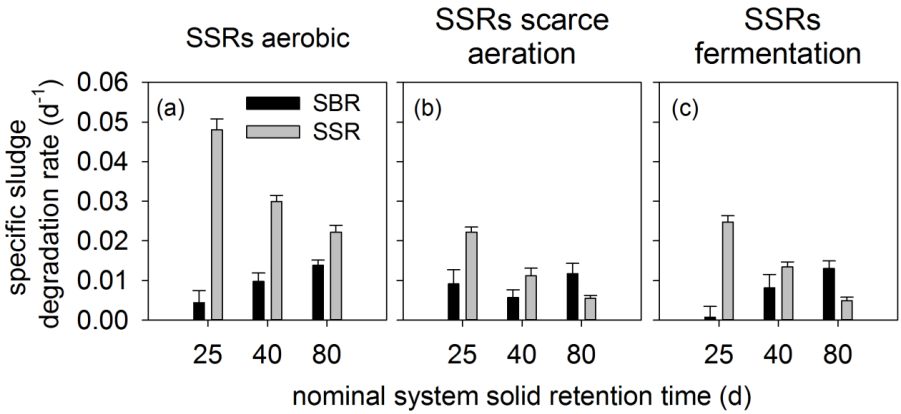


Fig. 2.4 Specific sludge degradation rates measured in the main reactors (SBRs) and side-stream reactors (SSRs). The rates are plotted in function of the nominal solid retention time of the treatment systems and the aeration conditions in the SSRs. Average values of whole experimental periods and corresponding standard error bars are shown.

Change of sludge composition by the SSRs

Characterisation of the sludge composition in terms of intracellular ATP, bound EPS, bound EPS carbohydrates and EPS proteins content was performed for the sludge of the SBRs and SSRs (Fig. 2.5). Since sludge is sequentially recycled between the SBRs and SSRs, comparing sludge composition provides information on processes inside the SSRs. The sequential recycling of the sludge between the SBRs and SSRs did not significantly influence their ATP

and EPS content. The composition of the sludge from the SBR and SSR were indeed similar for all operating conditions (Fig. 2.5). On average, the EPS content, in terms of proteins and carbohydrates, was 300 mg $gVSS^{-1}$ for all systems and conditions. The main constituents of the EPS (proteins and carbohydrates) were further analysed (Fig. 2.5). Overall, the protein to carbohydrate ratios were slightly lower in the sludge of the SSRs than in the sludge of the SBRs (Fig. 2.5). The differences between SBRs to SSRs in the ratios were most pronounced for the experimental phase with aerobic conditions in the SSRs (Fig. 2.5 (g)).

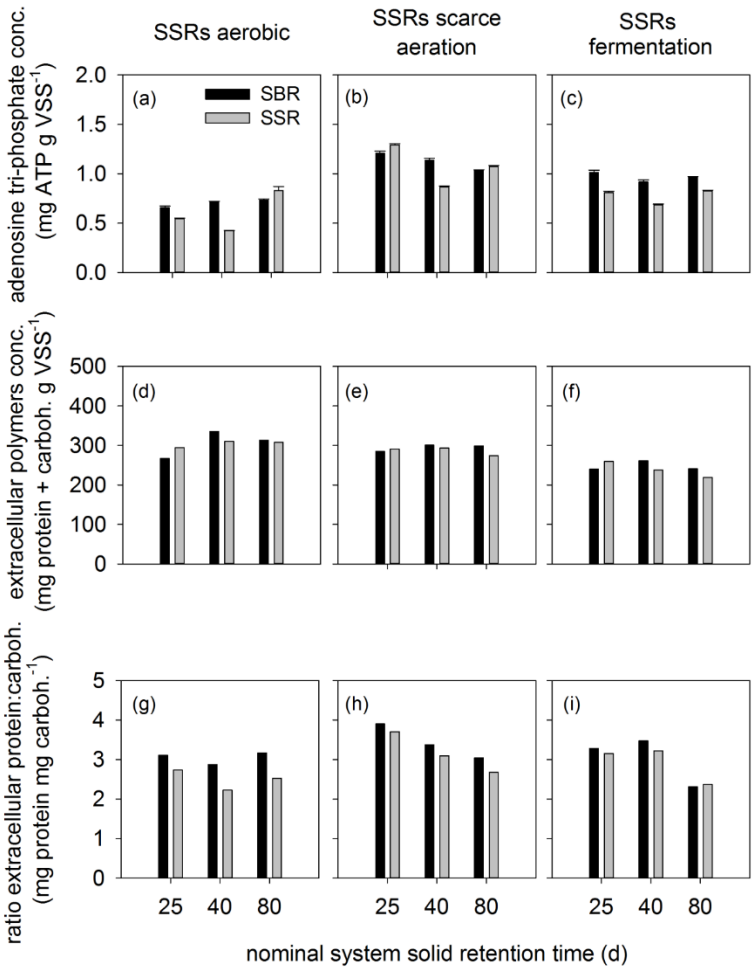


Fig. 2.5 Concentration of intracellular compounds (first row) and bound extracellular polymeric substances (EPS) (second row) in the sludge of the main reactors (SBRs) and side-stream reactors (SSRs). The third row compares ratios of extracellular proteins to carbohydrates in the EPS. Values are plotted as a function of the nominal solid retention time of the AS-SSR systems and of the aeration conditions in the SSRs. Error bars for adenosine tri-phosphate (ATP) show the standard error. For the extracellular polymeric substances in the sludge (second row) the method standard deviation was quantified as 9% of its average value, for the EPS proteins to carbohydrates ratio this value is 3% of the average.

Extracellular hydrolytic activities in the reactors

Different aeration conditions in the SSRs did not result in enhanced hydrolytic exoenzymatic activities in the SSRs (Fig. 2.6). The ratios of hydrolytic exoenzymatic activities of SSRs over SBRs were consistently close to 1 (Fig. 2.6, 1st row) or slightly smaller (Fig. 2.6, 2nd and 3rd row). Only at 80d SRT the hydrolytic exoenzymatic activities were consistently lower in the SSRs than in the SBRs (Fig. 2.6, 3rd row).

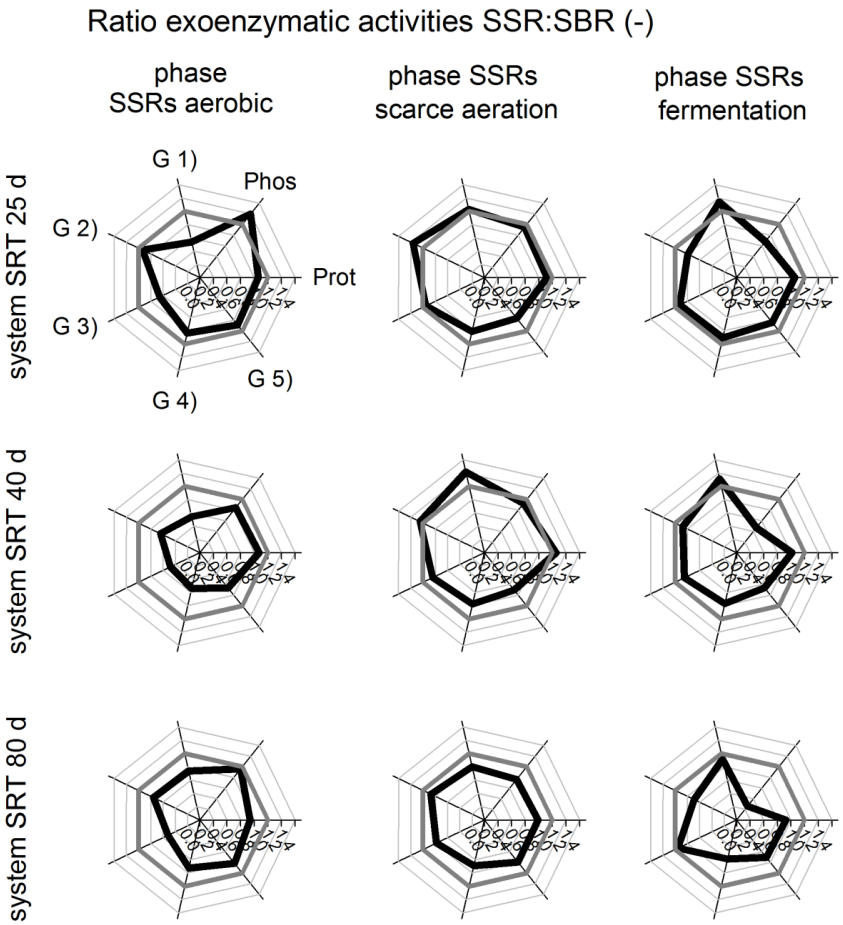


Fig. 2.6 Exoenzymatic activities expressed as the activity ratio of side-stream reactor (SSR) over the main reactor (SBR). The thick grey line represents equal activities in SSRs and SBRs (ratio of 1:1). The data is presented by the three experimental phases (columns) and by the wastewater treatment system (rows), SRT stands for solid retention time. The enzymatic activities plotted are: "Prot" Protease, "Phos" Phosphatase, several Glycosidases: "G 1)" β-N-acetylhexosaminidase, "G 2)" alpha-galactosidase, "G 3)" alpha-glucosidase, "G 4)" beta-galactosidase, "G 5)" beta glucosidase. The average standard error of the investigations is 2% of the mean value (individual values not displayable in this figure).

Effluent and sludge quality at long SRT

Effluent soluble COD values of the SBRs were between 10 and 20 mg COD L^{-1} (Fig. 2.7 (a)-(c), black bars). Effluent TSS values were between 5 and 14 mg L^{-1}. No obvious trends for different experimental phases or SRTs are recognized. The quality of the SBR sludge was characterized by sludge volume index (SVI_{30}) values between 40 and 70 mL g TSS^{-1} and the values of the SSRs were only slightly but consistently higher than that. High SRT did not deteriorate filterability of SBR sludge, contrary to the SSR sludge.

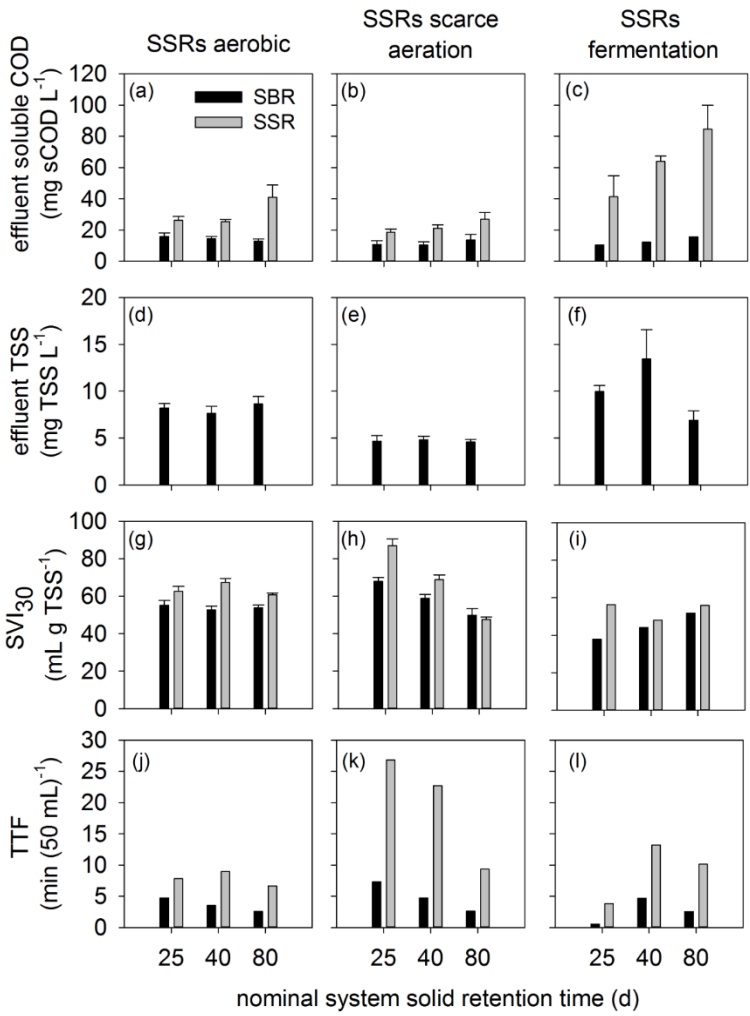


Fig. 2.7 Effluent and sludge quality indicators. Average data of effluent soluble carbonaceous chemical oxygen demand (sCOD) from the main reactors (SBRs) and the side-stream reactors (SSRs), effluent total suspended solids (TSS), sludge volume index after 30 minutes settling time (SVI_{30}) and dewaterability indicator "time-to-filter (TTF)" measuring the time necessary to filter 50 ml of sludge liquor. Error bars show the standard error. For graphs (j), (k), (l) no standard error is available but standard deviation of the method was quantified as 7% of the measurement value. For plot (c) – SBRs and whole graph (i) the number of measurements was not sufficient for standard error calculation.

Discussion

Effect of side-stream reactor operation and SRT on specific sludge degradation rates and observed sludge yield

The aim of this study was to better understand the impact of aeration conditions and SRT on sludge degradation. Indeed, contradicting observations were made in different studies on the effect of aeration conditions on sludge degradation. On the one hand anoxic and anaerobic conditions were reported to be key for sludge minimization (Chon et al., 2011; Coma et al., 2013; Johnson et al., 2008; Novak et al., 2007; Semblante et al., 2014). On the other hand, aerobic conditions were associated with highest sludge degradation rates in experimental studies (Siegrist et al., 1999) and modeling concept of ASM3 (Gujer et al., 1999). In our study, maintaining aerobic conditions in the SSRs led to highest specific sludge degradation rates (Fig. 2.4). Aerobic conditions in SSRs ultimately helped to significantly reduce the observed sludge yields (Y_{obs}), especially for AS-SSR systems operated at 40 and 80 d SRT (Fig. 2.3). For the 25 d SRT system, the effect of aeration conditions in the SSRs on the Y_{obs} was not evident. But our results also indicate that the Y_{obs} of AS-SSR systems operated at low SRT has a low sensitivity to the observed decrease of $b_{X,SSR}$. Many studies have focused on understanding the influence of a side-stream reactor on sludge production (Coma et al., 2013; Datta et al., 2009; Huang et al., 2014; Novak et al., 2007). However these studies compared systems with various experimental designs (e.g. SBR+digester, SBR with SSR) and/or with different SRTs, which renders the interpretation of the effect of the SSR on the sludge degradation difficult. The effect of aeration conditions on sludge degradation was however addressed in more details by Chon et al. (2011). In their study, Chon et al (2011) compared two identical AS-SSR systems that operated at similar overall SRTs (63 and 74 d, respectively). One SSR was operated under aerobic conditions while the other SSR was operated under anaerobic conditions. However, both conditions lead to similar Yobs. Our results as well as the study of Chon et al. (2011) clearly indicate that scarce aeration, fermentation and anaerobic conditions in SSRs did not enhance sludge degradation as compared to aerobic SSR conditions. However our observations are different from those of Chon et al. (2011) who reported similar Y_{obs} for aerobic and anaerobic conditions in the SSRs. Similar degradation rates in the aerobic and anaerobic SSRs

can be assumed by Chon et al. (2011) while they were significantly different in our study. Comparing our observations to those of Chon et al. (2011) provides relevant information about the design and operational parameters that promote sludge reduction using different aeration conditions. Similar parameters were used for the operation of the SSRs, listed in the following and indicating the applied specification by Chon et al. (2011) in brackets: mixing (continuously stirred), temperature (around 20 °C) and sludge mass distributed in SBRs vs. SSRs (60% vs. 40%). The HRTs in the SSRs were higher in our experiment with 12 - 20 days than compared to 10 days by Chon et al. (2011) which should however not make a large difference according to our own simulations with ASM3 (data not shown). Also our operation of the SSRs with occasional air injection to prevent methanogenesis was different to Chon et al. (2011) where no active control of metabolic activity was described in the anaerobic SSR. However allowing for fermentation but preventing methanogenesis in the way we did was successfully applied in Ramdani et al. (2012) for sludge reduction and can be found in full-scale operation (Johnson et al. 2008) and is therefore not thought to be a key difference. Other differences seem to play a role, since the operational mode of the SSRs are unable to explain contrasting findings between the study of Chon et al. (2011) and this work. Chon et al. (2011) operated the AS-SSR systems using i) a mixture of synthetic wastewater and primary effluent instead of only synthetic wastewater, ii) a lower influent soluble COD concentration of 351 mg sCOD L^{-1} as compared to 550 mg sCOD L^{-1} and iii) a wastewater injection presumably under aerobic conditions as compared to our anoxic conditions. Wastewater soluble COD and injection mode are discussed in the following section *active biomass degradation*. Influence of wastewater composition on sludge production and degradability is discussed in the following section *extracellular polymeric substances (EPS) hydrolysis*.

Processes in the side-stream reactors

Active biomass degradation

Rather similar concentrations of intracellular ATP per VSS were measured in the SBRs and SSRs (Fig. 2.5). The sludge from the SBR is the sludge that is flowing into the SSR, before side-stream digestion. The sludge from the SSR represents the side-stream digested sludge. Therefore, similar ATP per VSS values suggest that ATP and thus active biomass followed the degradation trend of VSS in the SSRs. This is supported by the approximated decay rate

coefficients of ATP indicating that active biomass degraded fastest under aerobic conditions overall (Fig. S 2.2). The fact that active biomass mainly decayed under aerobic but not under anaerobic conditions was recently observed for sludge from a full-scale AS-SSR plant (Foladori et al., 2015). That recent study and our results clearly indicate that exposing activated sludge to aerobic conditions is the most efficient strategy to decay active biomass. Another interesting and important finding is that even under aerobic conditions in the SSRs the active biomass decay was slower than expected. In our study, the SSRs were operated at long HRTs of 12-20 days. Based on an initial simulation with ASM3 (Gujer et al., 1999) with the corresponding default parameters the active biomass fractions (mg active biomass per mg VSS) were expected to decrease by 50 to 70% from the SBR sludge to the SSR sludge under aerobic SSR operation (detailed simulation data not shown). However the ATP measurements indicate that the concentrations of active biomass per VSS in the SSRs did not decline and were therefore similar in SBRs and SSRs. This holds the potential to explain why our Y_{obs} values for the aerobic SSR period were clearly higher than expected based on SRT dependent Y_{obs} prediction (Sperandio et al., 2013) A very low active biomass decay rate (0.025 d^{-1}) was recently also observed by Martínez-García et al. (2014). Specific operating conditions e.g., very high initial substrate concentrations under anaerobic conditions reduce the active biomass decay rate and this was attributed to the selection for storing microorganisms (Martínez-García et al., 2014). In our study high amounts of readily biodegradable substrate were injected within few minutes under anoxic conditions, similarly but not as extreme as in Martínez-García et al. (2014). Thus, the selection of specific microorganisms with slow decay rate might explain that similar active biomass concentrations were measured in the sludge of the SBR and SSR. Previous studies showed that short substrate injection durations can increase respectively decrease Y_{obs} of a system strongly, by 100% (Lobos et al., 2008) or up to 40% (Yagci et al., 2015), respectively. Also these latter observations indicate that wastewater treatment process type, including wastewater injection mode, influences sludge degradability and perhaps active biomass degradability. On the other hand it is not clear whether microbial storage is the key factor leading to low active biomass decay since sludge reduction was already applied in case of an enhanced biological phosphorus removal process (Huang et al., 2014).

Extracellular polymeric substances (EPS) hydrolysis

Contrary to active biomass, the bound EPS were expected to accumulate during side-stream digestion in the SSRs due to their slower biodegradability than active biomass (Laspidou and Rittmann, 2002). However, similar concentrations of EPS per VSS in the SBR and SSR sludge were measured in our study, around 300 mg proteins + carbohydrates per g VSS (Fig. 2.5). This value is lower than those measured by Ras et al. (2008) (401 mg g VSS^{-1}) for a sludge from synthetic wastewater treatment. This might come from differences in sludge ages (25 – 80 d in our study vs. 8 d in Ras et al. (2008)).

The similar EPS content measured in the SBR and SSR sludges indicates that EPS were degraded at a similar specific rate as the VSS. This is in line with the approximate hydrolysis rate coefficients of EPS which indicate that EPS degraded fastest under aerobic conditions (Fig. S 2.2). Highest specific aerobic EPS solubilization rates are contradicting other studies where enhanced EPS solubilization was attributed to anaerobic conditions (Foladori et al., 2015; Novak et al., 2007). In fact a common mechanism proposed to explain the enhanced sludge solubilization in AS-SSR systems with anaerobic conditions in SSRs is the hydrolysis of EPS, especially EPS proteins, through the reduction of floc-bound iron (Chon et al., 2011; Foladori et al., 2015; Johnson et al., 2008; Novak et al., 2007). This mechanisms was proposed based on batch-experiments performed by Park et al. (2006) and based on the work of Novak et al. (2003). However in the case of AS-SSR systems the occurrence of such mechanism was not evidenced and its contribution to the sludge reduction process was not proven. In our experiment this mechanism was not playing a major role. Under low redox potential conditions in the SSRs, including iron-reducing conditions in the fermentation mode, less EPS were hydrolyzed than under aerobic conditions. A further indication that this mechanism was absent is the fact that EPS proteins were not specifically attacked under fermentation conditions. This is indicated by EPS proteins to carbohydrates ratios (Fig. 2.5) that decreased strongest under aerobic conditions in the SSRs but little under fermentation conditions. In the current study the low iron concentration in the synthetic wastewater (Table 2.2) might explain why a low amount of sludge was degraded under fermentation conditions compared to aerobic conditions. A higher iron concentration in the influent could have increased the sludge degradation under fermentation conditions (Park et al., 2006).

Our observations indicate that the specific mechanism(s) leading to high degradation of EPS under fermentation or anaerobic conditions may only take place under favourable conditions. These favourable conditions may not only depend on iron, but generally on the inorganic cations present in the sludge floc matrix, defining the stability of activated sludge flocs and the EPS degradability (Novak et al., 2003). Since inorganic cations originate from the wastewater influent or from dosing, we can assume that the wastewater composition or dosing chemical composition influence the composition of sludge flocs in terms of cations. In addition it is not clear whether influent organics that can bind to sludge flocs would react differently from EPS (i.e. microbial polymers) when exposed to oxygen-limited degradation conditions. Thus, the reason why low redox potential conditions for sludge degradation were efficient in Chon et al. (2011) but not in our study could come from the different wastewater composition between that study and our experiment.

Extracellular enzymes could play an important role in degradation of particulate biomass and especially for EPS degradation, since hydrolysis of particulate organic matter is usually the rate limiting step in sludge degradation processes (Batstone et al., 2002). We observed that hydrolytic enzymatic activity did not change strongly between aerobic and scarce aeration or fermentation conditions in the SSRs (Fig. 2.6). This is in contrast to the specific sludge degradation rates in the SSRs that changed significantly in response to the aeration conditions in the SSRs (Fig. 2.4). This means that the strong decrease in the specific sludge degradation rates in the SSRs under scarce aeration and fermentation conditions was not caused by a disappearance of extracellular hydrolytic enzymatic activity. A fair stability of hydrolytic exoenzymes was already described under aerobic and anaerobic starvation by Goel et al. (1998), however only for short-term starvation. In that previous study, the observations on hydrolytic exoenzymes were not discussed in the context of endogenous sludge degradation activity. The missing link between hydrolytic exoenzymatic activity and specific sludge degradation rates in our investigation gives rise to following scenarios: i) another step than extracellular hydrolysis limited the rate of biomass degradation, such as predation by higher organisms or solubilization of polymers by different physico-chemical forces, ii) with the limited number of different hydrolytic exoenzymatic activities that were assessed in this study we could have missed specific hydrolytic enzymes that might be key for sludge degradation, such as lysozyme iii) the method for enzymatic activity measurement reflects the enzyme's activity under standardized

conditions, acting on well soluble substrate. This activity could differ from the in-situ activity in the reactors, acting on particulate substrate. The finding that hydrolytic exoenzymes embedded in sludge flocs are not limiting the rate of biomass degradation is interesting for analysing performance variations in AS-SSR systems. The fair stability of exoenzymes under different conditions suggests that failure of AS-SSR systems in terms of sludge reduction is not linked to missing hydrolytic exoenzymes, and thus fault analysis can focus on other parameters.

Use of long SRTs in sludge minimizing systems

Increasing the system SRT from high (25 d) to very high values (40 and 80 d) helped to increase the extent of sludge degradation. Operating activated sludge systems under long SRT conditions could potentially be detrimental for the systems performances, i.e., sludge properties and effluent quality. However, in our study the operation of the AS-SSRs at very long SRTs did not deteriorate the effluent quality or its settling and dewaterability properties (Fig. 2.7). The findings of our experiment confirm the strategy to reduce sludge by long SRT and that aerobic conditions might be most efficient in the solids turnover rate for at least some of these systems.

Conclusions

- Significant sludge reduction can be achieved in AS-SSR by increasing the overall SRT. Increasing the overall SRT from high (25 d) to very high (80 d) values helped to further decrease the Y_{obs} by up to 36%.
- Aerobic conditions in the SSRs lead to highest sludge degradation rates of VSS, active biomass and EPS. Reducing the availability of oxygen in the SSR decreases the degradation rates.
- EPS hydrolyzed with a similar specific rate as VSS under all aeration conditions. Therefore distinction of EPS from VSS for predicting the sludge degradability is not crucial.
- The activity of conventional hydrolytic exoenzymes embedded in sludge flocs, measured under ex-situ standardized conditions, is not a reliable indicator for the sludge degradability. The highest specific sludge degradation rates, measured for aerobic conditions, were not caused by generally increased hydrolytic exoenzymatic activities.

Supplementary Information

A) Calculation of $b_{X,SSR}$ and $b_{X,SBR}$

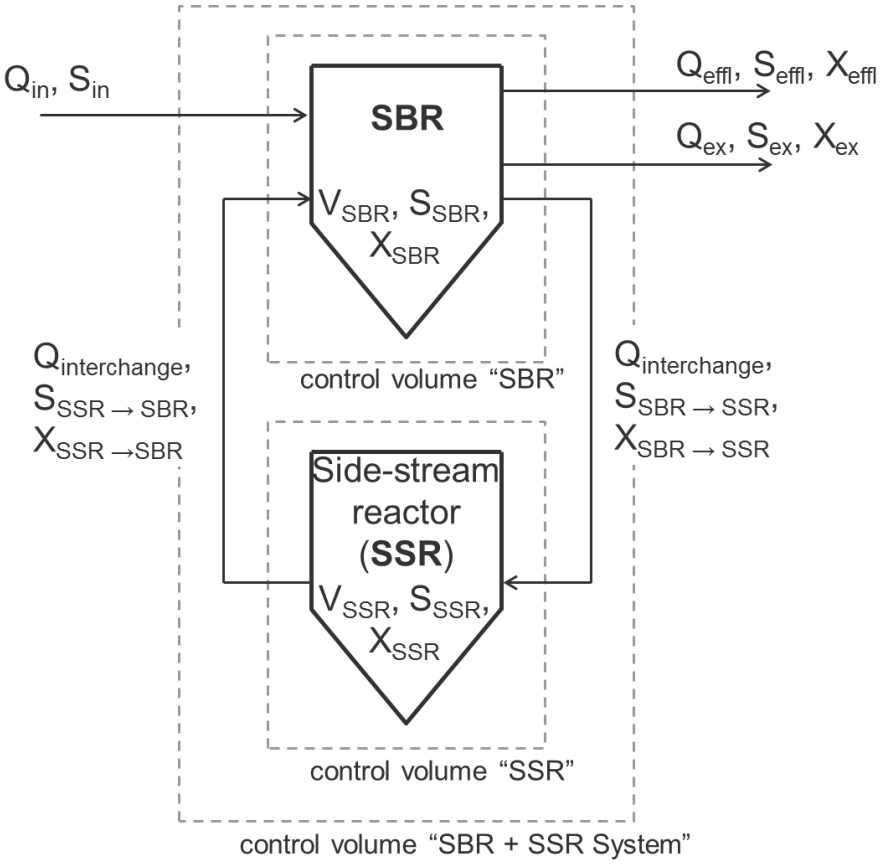


Fig. S 2.1 Scheme of one treatment system consisting of sequencing batch reactor (SBR) and side-stream reactor, concentrations for soluble (S) and particulate (X) compounds, volumes (V), and flow rates (Q) and three control volumes.

In the following, all solids mass (M_X), concentration (X) and rate coefficient (b_X) refer to solids unit mg volatile suspended solids (VSS). The specific sludge degradation rate for the SSR ($b_{X,SSR}$) is based on the solids mass balance on the control volume "SSR" (Fig. S 2.1). Following assumptions apply: the reactor is considered as completely mixed reactor and exogenous growth is negligible. The solids degradation rate is represented by a first order rate.

$$\frac{dM_{X,SSR}}{dt} = \frac{d(V_{SSR} * X_{SSR})}{dt} = Q_{interchange} * (X_{SBR \rightarrow SSR} - X_{SSR \rightarrow SBR}) - b_{X,SSR} * X_{SSR} * V_{SSR}$$

(S 2.1)

$dM_{X,SSR}\ dt^{-1}$: solids accumulation rate in the SSR (mg VSS d^{-1})

V_{SSR}: Volume of the SSR (L)

X_{SSR}: solids concentration in the SSR (mg VSS L^{-1})

$Q_{interchange}$: Interchange flow rate (L d^{-1})

$X_{SBR \rightarrow SSR}$: solids concentration in the interchange flow towards the SSR (mg VSS L^{-1})

$X_{SSR \rightarrow SBR}$: solids concentration in the interchange flow towards the SBR (mg VSS L^{-1})

$b_{X,SSR}$: specific sludge degradation rate of the SSR (d^{-1})

Eq. (S 2.1) can be rearranged to separate $b_{X,SSR}$ and then be calculated. For the calculation of $b_{X,SSR}$ the accumulation rate of sludge in the SSR over one or two weeks ($\Delta(V_{SSR} * X_{SSR})\ \Delta t^{-1}$) was considered rather than over an infinitesimal time period ($d(V_{SSR} * X_{SSR})\ dt^{-1}$).

$$b_{X,SSR} = \left(Q_{interchange} * (X_{SBR \rightarrow SSR} - X_{SSR \rightarrow SBR}) - \frac{\Delta(V_{SSR} * X_{SSR})}{\Delta t} \right) * \frac{1}{X_{SSR} * V_{SSR}}$$

(S 2.2)

The specific sludge degradation rate for the SBR ($b_{X,SBR}$) is based on the solids mass balance of the overall system, see control volume "SBR + SSR System" (Fig. S 2.1). The considered processes are (i) the growth of heterotrophs and autotrophs on exogenous substrate, (ii) the solids degradation in the SBR and (iii) the solids degradation in the SSR, both represented by a first order degradation rate, and (iv) the solids that are transported out of the system.

$$\frac{dM_{X,SBR+SSR}}{dt} = R_{growth,X} - b_{X,SBR} * X_{SBR} * V_{SBR} - b_{X,SSR} * X_{SSR} * V_{SSR} - J_X$$

(S 2.3)

$dM_{X,SBR+SSR}\ dt^{-1}$: solids accumulation rate in the system (mg VSS d^{-1})

$R_{growth,X}$: solids growth rate (mg VSS d^{-1})

$b_{X,SBR}$: specific sludge degradation rate of the SBR (d^{-1})

X_{SBR}: solids concentration in the SBR (mg VSS L^{-1})
V_{SBR}: Volume of the SBR (L)
J_X: solids transport rate of out of the system (mg VSS d^{-1})

The solids mass flow rate (J) is the sum of the excess solids mass flow rate and the effluent solids mass flow rate.

$$J_X = Q_{ex} * X_{ex} + Q_{effl} * X_{effl} \quad \text{(S 2.4)}$$

Q_{ex}: excess sludge flow rate (L d^{-1})
X_{ex}: solids concentration in excess sludge flow (mg VSS L^{-1})
Q_{effl}: effluent flow rate (L d^{-1})
X_{effl}: solids concentration in the effluent (mg VSS L^{-1})

Growth of heterotrophic and autotrophic biomass on soluble exogenous substrate occurs almost exclusively in the SBR. Therefore only the growth processes in the SBR are considered.

$$R_{growth,X} = R_{growth,X,OHO} + R_{growth,X,ANO} \quad \text{(S 2.5)}$$

$R_{growth,X,OHO}$: heterotrophs growth rate (mg VSS d^{-1})
$R_{growth,X,ANO}$: autotrophs growth rate (mg VSS d^{-1})

For heterotrophs, the growth rate can be estimated by the COD conversion rate, i.e. the rate by which soluble substrate is converted into CO_2 and solids, times the heterotrophs growth yield and the solids conversion factor (COD to VSS). The growth rate of nitrifiers is calculated similarly as for heterotrophs, but considering the production rate of nitrate instead of the COD conversion rate. This calculation way is deduced from common model structure such as ASM (Henze et al. 2000). Since part of the produced nitrate was transformed to nitrogen gas by denitrification in the SBRs, this part is explicitly considered in the following. For the ordinary

heterotrophic organisms (X_{OHO}) and the autotrophic nitrifying organisms (X_{ANO}) the growth rate can thus be expressed as:

$$R_{growth,X,OHO} = -R_{transformation,sCOD} * Y_{OHO} * \frac{1}{f_{CV,X,Bio}} \quad \text{(S 2.6)}$$

$$R_{growth,X,AUT} = R_{formation,N-nitrified} * Y_{AUT} * \frac{1}{f_{CV,X,Bio}} \quad \text{(S 2.7)}$$

$R_{transformation,sCOD}$: soluble substrate conversion rate into CO_2 and solids (mg COD d^{-1})
Y_{OHO}: growth yield of heterotrophs (mg COD mg COD^{-1})
$f_{CV,X,Bio}$: conversion factor COD to VSS for active biomass (mg COD mg VSS^{-1})
$R_{formation,N-nitrified}$: nitrified-nitrogen formation rate (mg NO_3-N d^{-1} or N_2-N d^{-1})
Y_{ANO}: growth yield of autotrophs (mg COD mg NO_3-N^{-1} or mg COD mg N_2-N^{-1})

Growth yields are taken over from ASM1 (Henze et al. 2000), for heterotrophs Y_{OHO} = 0.67 mg COD mg COD^{-1} and for autotrophs Y_{ANO} = 0.24 mg COD mg NO_3-N^{-1} or mg COD mg N_2-N^{-1}. A typical conversion yield of $f_{CV,X,Bio}$ of 1.42 mg COD mg VSS^{-1} is assumed (Spérandio et al. 2013).
The soluble substrate conversion rate for COD is deduced from the mass balance of soluble compounds over the control volume of the SBR (control volume "SBR", Fig. S 2.1):

$$\frac{dM_{S,COD,SBR}}{dt} = \frac{d(S_{COD} * V_{SBR})}{dt} = Q_{in} * S_{COD,in} + Q_{interchange} * (S_{COD,SSR \rightarrow SBR} - S_{COD,SBR \rightarrow SSR}) - (Q_{ex} * S_{COD,ex} + Q_{effl} * S_{COD,effl}) + R_{transformation,S,COD} \quad \text{(S 2.8)}$$

$dM_{S,COD,SBR}\ dt^{-1}$: soluble COD accumulation rate in the SBR (mg COD d^{-1})

S_{COD}: soluble COD concentration in the SBR (mg COD L^{-1})

Q_{in}: influent wastewater flow rate (L d^{-1})

$S_{COD,in}$: soluble COD concentration in the influent (mg COD L^{-1})

$S_{COD,SSR \rightarrow SBR}$: soluble COD concentration in the sludge interchange flow arriving from the SSR (mg COD L^{-1})

$S_{COD,SBR \rightarrow SSR}$: soluble COD concentration in the sludge interchange flow leaving the SBR (mg COD L^{-1})

$S_{COD,ex}$: soluble COD concentration in the excess sludge flow (mg COD L^{-1})

$S_{COD,effl}$: soluble COD concentration in the effluent (mg COD L^{-1})

$R_{transformation,S,COD}$: soluble COD transformation rate (mg COD d^{-1})

Following simplifications apply (i) the soluble COD concentrations in the SBR at the beginning and at the end of each cycle are very similar, therefore the soluble COD accumulation over long term in the SBRs is very close to zero (ii) the soluble COD mass exchanged by the sludge interchange flow between the SBR and the SSR is negligible in comparison to the influent COD load (iii) the soluble substrate concentrations in the effluent and in the excess sludge removal flow are very similar and thus considered to be equal, (iv) the influent flow rate equals the sum of effluent and excess sludge removal flow rates. Considering (i) - (iv) the soluble substrate transformation rate can be rearranged to

$$R_{transformation,S,COD} = Q_{in} * (S_{COD,effl} - S_{COD,in})$$

(S 2.9)

The formation rate of nitrified nitrogen (i.e. nitrogen under the form of NO_3^- and N_2) is calculated based on the soluble compounds mass balance as well, according to the same principles as soluble COD. The resulting expression is

$$R_{formation,N-nitrified} = Q_{in} * \left((S_{NO3-N,effl} - S_{NO3-N,in}) + (S_{N2-N,effl} - S_{N2-N,in})\right)$$

(S 2.10)

$S_{NO3\text{-}N,effl}$: nitrate-nitrogen concentration in the effluent (mg NO_3-N L^{-1})

$S_{NO3\text{-}N,in}$: nitrate-nitrogen concentration in the influent (mg NO_3-N L^{-1})
$S_{N2\text{-}N,effl}$: nitrogen gas – nitrogen concentration in the effluent (mg N_2-N L^{-1})
$S_{N2\text{-}N,in}$: nitrogen gas – nitrogen concentration in the influent (mg N_2-N L^{-1})

For simplicity, denitrified nitrogen is described as a soluble phase in eq. (S 2.10). In the actual calculations, the nitrogen gas effluent concentration is deduced from the disappearance of NO_3-N during the anoxic filling phase, which was measured for each SBR individually.

Influent nitrate and nitrogen gas concentration are very close to zero, thus eq. (S 2.10) can be simplified to

$$R_{formation,N-nitrified} = Q_{in} * (S_{NO3-N,effl} + S_{N2-N,effl}) \quad \text{(S 2.11)}$$

Insertion of eq. (S 2.4 – S 2.11) into eq. (S 2.3), gives an expression based on measurable values:

$$\frac{dM_{X,SBR+SSR}}{dt} = Q_{in} * \left(S_{COD,in} - S_{COD,effl}\right) * Y_{OHO} * \frac{1}{f_{CV,X,Bio}} + Q_{in} * \left(S_{NO3-N,effl} + S_{N2-N,effl}\right) * Y_{ANO} * \frac{1}{f_{CV,X,Bio}} - (b_{X,SBR} * X_{SBR} * V_{SBR} + b_{X,SSR} * X_{SSR} * V_{SSR}) - (Q_{ex} * X_{ex} + Q_{effl} * X_{effl}) \quad \text{(S 2.12)}$$

The specific sludge degradation rate of the SBR ($b_{X,SBR}$) can be separated in eq. (S 2.12). The actual calculations considered an accumulation rate of sludge in the system over one or two weeks ($\Delta M_{X.SBR+SSR}\ \Delta t^{-1}$) rather than over an infinitesimal time period ($dM_{X.SBR+SSR}\ dt^{-1}$).

$$b_{X,SBR} = \left(Q_{in} * \left(S_{COD,in} - S_{COD,effl}\right) * Y_{OHO} * \frac{1}{f_{CV,X,Bio}} + Q_{in} * \left(S_{NO3-N,effl} + S_{N2-N,effl}\right) * Y_{ANO} * \frac{1}{f_{CV,X,Bio}} - b_{X,SSR} * X_{SSR} * V_{SSR} - \left(Q_{ex} * X_{ex} + Q_{effl} * X_{effl}\right) - \frac{\Delta M_{X,SBR+SSR}}{\Delta t} \right) * \frac{1}{X_{SBR} * V_{SBR}} \quad \text{(S 2.13)}$$

B) Calculation of sensitivity of Y_{obs} towards $b_{X,SSR}$

The sensitivity of Y_{obs} to the value of $b_{X,SSR}$ was also estimated. For a **single reactor** activated sludge tank the steady-state Y_{obs} can be predicted by an analytical expression (Tchobanoglous et al. 2003). Assuming a first order total solids degradation rate and no influent unbiodegradable COD the following expression results.

$$Y_{obs,model} = Y_{OHO} * \frac{1}{f_{CV,X,Bio}} * \frac{1}{1 + SRT * b_X} \quad \text{(S 2.14)}$$

$Y_{obs,model}$: observed sludge yield, modelled (mg VSS mg COD^{-1})
SRT: solid retention time (d)
b_X: specific sludge degradation rate (d^{-1})

In order to get an improved estimation of the $Y_{obs,model}$ for a **two-reactor** system, two steps were carried out. First, the specific sludge degradation rate (b_X) in eq. (S 2.14) was described as a function of $b_{X,SBR}$ and $b_{X,SSR}$, weighed proportional to the mass in the corresponding reactors.

$$b_X = \frac{b_{X,SBR} * X_{SBR} * V_{SBR} + b_{X,SSR} * X_{SSR} * V_{SSR}}{X_{SBR} * V_{SBR} + X_{SSR} * V_{SSR}} \quad \text{(S 2.15)}$$

Second, a correction factor "η" was introduced to take into account that our two reactor system would generally produce more sludge than a single-reactor system. This is because we remove "young" waste sludge from the SBR rather than "old" sludge from the SSR. This correction factor is added as a linear factor.

$$Y_{obs,model} = Y_{OHO} * \frac{1}{f_{CV,X,Bio}} * \frac{1}{1 + SRT * b_X} * n \quad \text{(S 2.16)}$$

To estimate the sensitivity of $Y_{obs,model}$ regarding $b_{X,SSR}$, η was first calibrated so that $Y_{obs,model}$ would match measured observed sludge yields (Y_{obs}) of the experimental phase "SSRs aerobic". Then $Y_{obs,model}$ for the two other experimental phases, "SSRs scarce aeration" and "SSRs fermentation" were calculated, with the experimentally determined $b_{X,SSR}$ values. Further parameters used for $Y_{obs,model}$ calculation of the scarce aeration and fermentation conditions were (i) constant: Y_{OHO} = 0.67 mg COD mg COD^{-1}, $f_{CV,X,Bio}$ = 1.42 mg COD mg VSS^{-1}, SRT of phase "SSRs aerobic", $b_{X,SBR}$ of experimental phase "SSRs aerobic", η calibrated for phase "SSRs aerobic" and (ii) individual: $b_{X,SSR}$, X_{SSR}, V_{SSR}, X_{SBR}, V_{SBR} for the experimental phases "SSRs scarce aeration" and "SSRs fermentation".

C) Calculation of intracellular ATP decay and EPS hydrolysis coefficients

Coefficients of ATP decay and EPS hydrolysis for the SSRs were estimated using a first order degradation rate equation. The calculations were based on the ATP and EPS measurements normalized by the VSS concentrations (Fig. 2.5). The SSRs were considered as continuous flow stirred tank reactors (CSTRs) working with the average sludge reduction performance, i.e. characterized by the average $b_{X,SSR}$ (eq. (2.1)) of each experimental phase (Fig. 2.4), under steady-state operation (Tchobanoglous et al. 2003). The basic variables are displayed in Fig. S 2.1.

$$b_{ATP} = \frac{1}{\tau}\left(\frac{\left(\frac{X_{ATP,SBR\rightarrow SSR}}{X_{SBR\rightarrow SSR}}\right)\cdot X_{SBR\rightarrow SSR}}{\left(\frac{X_{ATP,SSR}}{X_{SSR}}\right)\cdot X_{SSR}} - 1\right) \quad \text{(S 2.17)}$$

b_{ATP}: first order decay rate coefficient for intracellular ATP (d^{-1})

τ: hydraulic residence time in the SSR (d)

($X_{ATP,SBR\rightarrow SSR}\ X_{SBR\rightarrow SSR}^{-1}$): measured ratio of intracellular ATP over VSS in the influent of the SSR (mg ATP g VSS^{-1})

($X_{ATP,SSR}\ X_{SSR}^{-1}$): measured ratio of intracellular ATP over VSS in the SSR (mg ATP g VSS^{-1})

$X_{SBR\rightarrow SSR}$: VSS concentration in the influent towards the SSR at steady-state conditions (g VSS L^{-1})

X_{SSR}: VSS concentration in the SSR at steady-state conditions (g VSS L^{-1})

The corresponding equation for the EPS hydrolysis coefficient:

$$b_{EPS} = \frac{1}{\tau}\left(\frac{\left(\frac{X_{EPS,SBR\rightarrow SSR}}{X_{SBR\rightarrow SSR}}\right)\cdot X_{SBR\rightarrow SSR}}{\left(\frac{X_{EPS,SSR}}{X_{SSR}}\right)\cdot X_{SSR}} - 1\right)$$

(S 2.18)

b_{EPS}: first order hydrolysis rate coefficient for EPS (d^{-1})

($X_{EPS,SBR \rightarrow SSR}$ $X_{SBR \rightarrow SSR}^{-1}$): measured ratio of EPS over VSS in the influent of the SSR (mg ATP g VSS^{-1})

($X_{EPS,SSR}$ X_{SSR}^{-1}): measured ratio of EPS over VSS in the SSR (mg ATP g VSS^{-1})

D) Sludge degradation and sludge inventory in the SBR vs. SSR

Table S 2.1 : Measured performance in terms of sludge degradation rate (g VSS d^{-1}) in the individual reactors and sludge mass (sludge inventory) in the systems. Average values are presented for the different experimental phases.

		Nominal SRT and reactor					
		"25 d SRT"		"40 d SRT"		"80 d SRT"	
Parameter	Experimental conditions	SBR	SSR	SBR	SSR	SBR	SSR
Sludge degradation rate (g VSS d^{-1})	SSRs aerobic	0.96	0.91	1.16	0.96	1.51	1.13
	SSRs scarce aeration	1.14	0.61	1.00	0.52	1.69	0.54
	SSRs fermentation	0.88	0.69	1.13	0.66	1.67	0.45
Sludge inventory (g VSS)	SSRs aerobic	30	19	37	33	49	58
	SSRs scarce aeration	36	28	42	47	66	97
	SSRs fermentation	35	28	43	49	64	93

E) Intracellular ATP decay and EPS hydrolysis coefficients in the SSRs

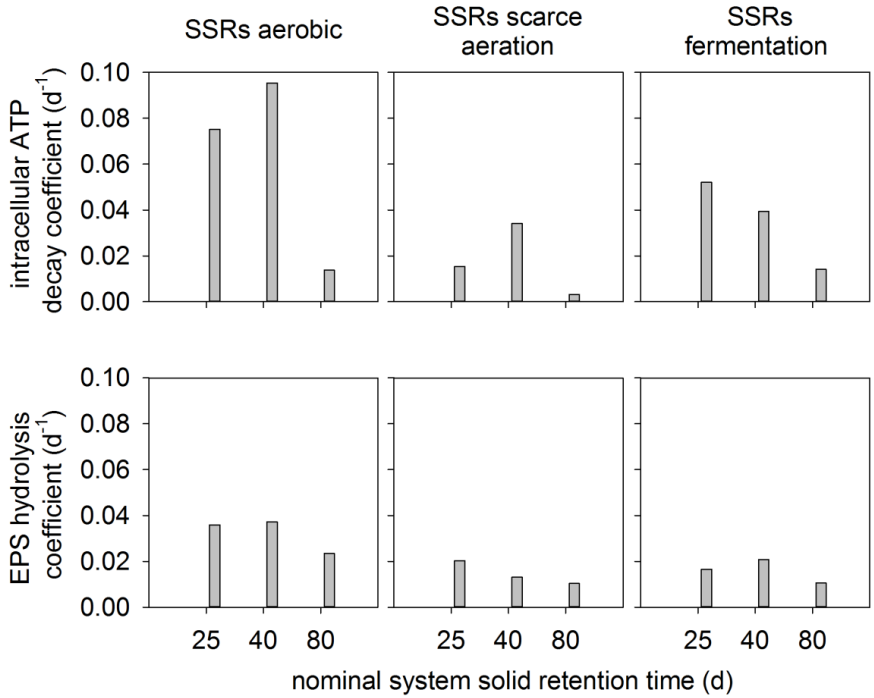


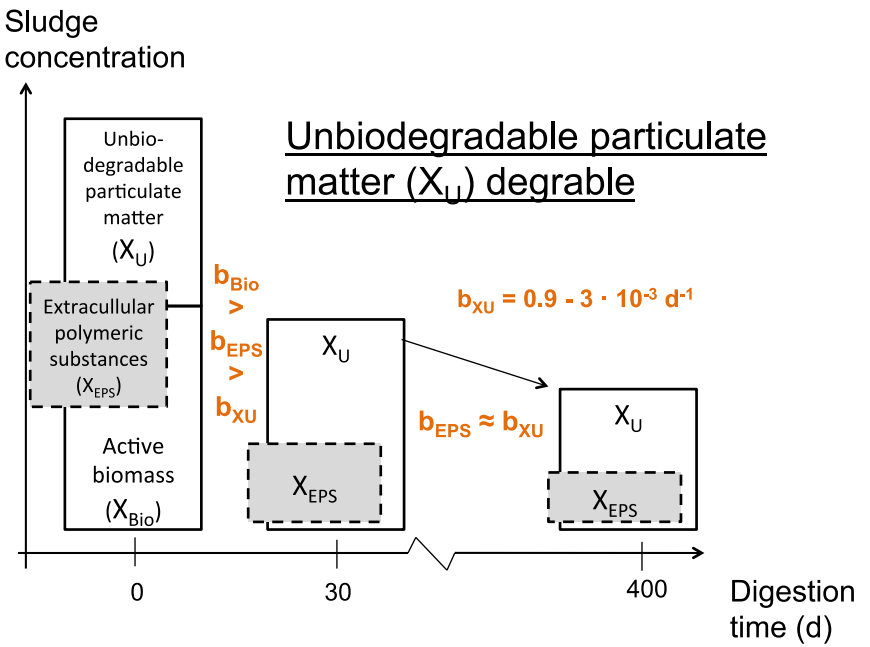
Fig. S 2.2 Intracellular ATP and EPS decay coefficients by different experimental periods and by the different experimental systems.

Chapter 3

Degradation of the unbiodegradable particulate fraction (X_U) from different activated sludges during batch digestion tests at ambient temperature

Habermacher, J., Benetti, A.D., Derlon, N. and Morgenroth, E. (2016) Degradation of the unbiodegradable particulate fraction (XU) from different activated sludges during batch digestion tests at ambient temperature. Water Research 98, 206-214. DOI: 10.1016/j.watres.2016.04.005

Graphical Abstract



Highlights

- Biodegradability of the unbiodegradable particulate fraction (X_U) was studied
- X_U produced from real wastewater degraded under conditions relevant for on-site WWT
- X_U degradation rate coefficients (b_{XU}) of 0.7 to 1.7 ·10^{-3} d^{-1}
- Overall bound EPS are degradable and do not accumulate in X_U
- Cation bound EPS proteins degraded slower than hydrophobic bound EPS proteins

Abstract

One strategy for the management of excess sludge in small wastewater treatment plants (WWTPs) consists in minimizing the excess sludge production by operating the WWTP at very long solids retention times (SRTs > 30 days). A number of recent studies have suggested that sludge minimization at very long SRT results from the degradation of the unbiodegradable particulate fraction (X_U) (influent unbiodegradable compounds and endogenous decay products). But the biodegradability of the unbiodegradable particulate fraction has only been evaluated during batch digestion test performed at ambient temperature with sludge fed with synthetic wastewaters. It is not clear to what extent observations made for sludge fed with synthetic influents can be transposed to sludge fed with real influent. The current study thus focused on evaluating the biodegradability of the unbiodegradable particulate fraction for sludge fed with real wastewater. Batch digestion tests (400 days, ambient temperature) were conducted with three different sludges fed with either synthetic or real influents and exposed to aerobic or intermittent aeration conditions. Our results indicate that volatile suspended solids (VSS) decreased even after complete decay of the active biomass (i.e., after 30 days of aerobic batch digestion) indicating that the unbiodegradable particulate fraction is biodegradable. However, very low degradation rates of the unbiodegradable particulate fraction were monitored after day 30 of digestion ($0.7\text{-}1.7 \cdot 10^{-3}$ d^{-1}). These values were in the lower range of previously published values for synthetic wastewaters ($1 - 7.5 \cdot 10^{-3}$ d^{-1}). The low values determined in our study indicate that the rate could decrease over time or that sludge composition influences the degradability of the unbiodegradable particulate fraction. But our results also demonstrate that extracellular polymeric substances (EPS) have a minor impact on the biodegradability of the unbiodegradable particulate fraction. Overall bound EPS were indeed biodegradable under all conditions and thus did not accumulate in the unbiodegradable particulate fraction. Different bound EPS pools (e.g., cation bound EPS) were associated with specific degradation behaviors. Besides improved mechanistic understanding of sludge degradation processes, our results have implications for the development of decentralized wastewater treatment technologies with on-site reduction of excess sludge.

Keywords

unbiodegradable particulates, bound EPS, active biomass, aerobic digestion.

Nomenclature

overall bound EPS	sum of bound EPS proteins plus bound EPS carbohydrates
bound EPS proteins	sum of proteins extracted from sludge by serial extraction
bound EPS carbohydrates	sum of carbohydrates extracted from sludge by serial extraction
b_{VSS} (d^{-1})	degradation rate coefficient for VSS, first order
b_{EPS} (d^{-1})	degradation rate coefficient for overall bound EPS, first order
b_{XU} (d^{-1})	degradation rate coefficient for the unbiodegradable particulate fraction, first order
$b_{XU,E}$ (d^{-1})	degradation rate coefficient for endogenous residues, first order
X_U	unbiodegradable particulate fraction
$X_{U,Inf}$	influent unbiodegradable particulate fraction

Introduction

Sludge management for small scale decentralized wastewater treatment plants (WWTPs) requires the development of specific technologies. A relevant technology is the on-site sludge minimization. On-site sludge minimization can be achieved by increasing the solids retention time (SRT) (Spérandio et al. 2013). However, increasing the SRT increases the unbiodegradable particulates content of the sludge. The successful minimization of sludge exposed to long SRT conditions thus requires understanding to what extent and at which kinetic the unbiodegradable particulate fraction is biodegradable.

Biodegradability of the endogenous residues. The application of very long SRT for sludge minimization is only possible if the unbiodegradable particulate fraction (X_U) is biodegradable (Ramdani et al. 2012a, Spérandio et al. 2013). The literature does not provide a clear definition of "very long SRT". Very long SRT is considered as the SRT beyond which no further solids degradation is predicted by conventional models (e.g. ASM models). Values for very long SRT thus change with the type of treatment; from 30 days for batch digestion (Ramdani et al. 2012b) to more than 100 days for MBRs (Spérandio et al. 2013). Several studies suggested that the unbiodegradable particulate fraction is biodegradable under anaerobic conditions at 35 - 37 °C (Jones et al. 2008, Jones et al. 2009, Ramdani et al. 2010), with exception of Ekama et al. (2007). However, there is no evidence that the unbiodegradable particulate fraction is also biodegradable at ambient temperature and under oxic conditions, i.e., under operating conditions that are relevant for sludge minimization in small scale decentralized WWTPs. The biodegradability of the unbiodegradable particulate fraction was also suggested in MBRs operated at very long SRTs (Spérandio et al. 2013). In MBRs, the degradation of the unbiodegradable particulate fraction could be driven by the presence of active biomass. If active biomass is key for degrading the unbiodegradable particulate fraction, it is likely that the unbiodegradable particulate fraction degrades slower in a post digestion process. Another process operated at SRTs up to 400 days is the activated sludge – anaerobic side-stream reactor (Labelle et al. 2015). But for such process it remains unclear to what extent the unbiodegradable particulate fraction is biodegradable (Johnson et al. 2008, Menniti et al. 2012). Finally, degradation of the unbiodegradable particulate fraction was reported at ambient temperature (20 °C) and aerobic or intermittent aerated conditions in digestion mode by Ramdani et al.

(2012a) and Martínez-García et al. (2016). Even though such operating conditions are relevant for small scale decentralized WWTPs, the studies of Ramdani et al. (2012a) and Martínez-García et al. (2016) were performed with activated sludge grown on acetate as the sole carbon source. Getting evidences of the biodegradability of the unbiodegradable particulate fraction for sludge fed with real wastewater and digested under longer digestion times at ambient temperatures is thus still required.

Degradability of overall bound EPS. Activated sludge flocs contain large amounts of bound EPS, up to 40% (Ramdani et al. 2012b). Therefore the degradation of bound EPS is an important process in sludge minimization. The biodegradability of overall bound EPS decreases over the time of digestion, as summarized in the following. High degradation rates for overall bound EPS were indeed measured in short term experiments (less than two days). Degradation rate coefficients (b_{EPS}) of around 0.12 d^{-1} (Menniti and Morgenroth 2010) and 0.17 d^{-1} (Laspidou and Rittmann 2002a) were reported. These values of b_{EPS} are in the same order of magnitude than the decay coefficients of heterotrophic biomass e.g., 0.24 d^{-1} (Dold 2007). Bound EPS thus seem fairly biodegradable during the first days of digestion. However, bound EPS can have a heterogeneous arrangement within flocs and therefore different degradation kinetics (Ramdani et al. 2012b). Lower values of b_{EPS} were indeed observed by Ramdani et al. (2012b) during 21d batch digestion: b_{EPS} of 0.07 and 0.06 d^{-1} were quantified for activated sludge with SRT of 5.2 and 10.4 d, respectively (back-calculated from Ramdani et al. (2012b)). In a system with very high sludge ages of 25 to 80 days and aerobic digesters operated with long HRTs of 12 - 20 days, Habermacher et al. (2015) measured b_{EPS} value of 0.02 to 0.04 d^{-1}. The dynamic of overall bound EPS is thus determined up to digestion times of 20 days. However it remains unclear whether overall bound EPS are biodegradable at very long retention times or if they accumulate within residual sludge during digestion.

Few studies also indicate that the different pools of overall bound EPS have distinct degradabilities. Thus, the biodegradability of overall bound EPS could depend on the initial EPS composition. The pools of bound EPS can be classified according to the polymeric class, e.g. proteins or carbohydrates, and according to the method used for their extraction. Different methods are employed for the extraction of polymers with specific binding-properties from sludge or biofilms (Comte et al. 2007, Park and Novak 2007, Park et al. 2008, Ras et al. 2011).

Bound EPS proteins degrade faster than bound EPS carbohydrates at ambient temperature (Habermacher et al. 2015, Ramdani et al. 2012b). But these studies were performed using a single type of sludge that was fed with a synthetic wastewater. Park and Novak (2007) showed that EPS proteins bound to iron degraded at a higher rate than EPS proteins bound by other mechanisms to the flocs during anaerobic digestion. All these studies thus provide relevant insights about the link between the type of bound EPS and its degradability rate. But it is unclear whether all pools of bound EPS are biodegradable, or if non-biodegradable pools of EPS accumulate in the unbiodegradable particulate fraction of sludge over time.

Objectives. This study addressed the following questions: (i) how biodegradable is the unbiodegradable particulate fraction from real activated sludge at ambient temperature and under full or intermittent aeration in absence of important amounts of active biomass? (ii) are bound EPS accumulating during digestion because of decreasing degradability? Batch digestion tests with sludge from three different sources were thus conducted at ambient temperature, with full and intermittent aeration and during an extended period of 400 days. The degradation of the different sludge fractions (intracellular adenosine-triphosphate (ATP), bound EPS, organics attributed to the unbiodegradable particulate fraction) were monitored.

Materials and methods

Activated sludges

Three different sludges were produced and subsequently digested (Table 3.1). We hypothesized that (i) the initial composition of the sludge in terms of EPS content governs the composition of the unbiodegradable particulate fraction that accumulates at long SRT and that (ii) the composition of the unbiodegradable particulate fraction in turn determines its biodegradation rate, i.e., its decay coefficient b_{XU}. To test these hypotheses three different sludges were digested under different aeration conditions for a period of 400 d: (i) activated sludge from real wastewater (ii) activated sludge from synthetic wastewater, (iii) batch biomass from a batch growth culture. We assumed that the different growth histories of the sludges determined their initial composition.

Table 3.1 : Details of the sludges used in the digestion experiment and of the digestion characteristics. HRT=Hydraulic retention time, COD=Chemical oxygen demand, COD:N:P=Ratio of influent COD to Nitrogen to Phosphorus.

	Sludge production							Sludge digestion
Sludge name	Origin of sludge	Solids ret. time (d)	HRT (d)	Influent COD conc. ($mg\,L^{-1}$)	COD sources (load-contribution)	Influent COD : N : P (-)	Environ-mental conditions	Experiment number and condition
Activated sludge from real wastewater	Sequencing batch reactor treating primary effluent, filtered at 100 µm	5	0.6	Total COD : 380 Soluble COD : 220	Primary effluent organics (100%)	100/13/3	Aerobic	# 1 full aeration # 2 intermittent aeration
Activated sludge from synthetic wastewater	Sequencing batch reactors treating synthetic wastewater at high solid retention times	37	0.6	Soluble COD(*) : 550	Peptone (42%); Yeast extract, hydrolyzed (32%); Acetate (26%)	100/15/3	Anoxic (filling) – aerobic (reaction)	# 3 full aeration # 4 intermittent aeration
Batch biomass	Batch reactor with synthetic growth media, seeded with tiny amounts of activated sludge	40 (cultiv ation time)	40	Soluble COD(*) : 4600 (initial bulk conc.)	Glucose (60%) Acetate (40%)	100/20/5	Permanent aeration	# 5 full aeration # 6 intermittent aeration

(*) the influent of "Activated sludge from synthetic wastewater" and from "Batch biomass" contained only soluble COD.

Activated sludge from real wastewater

The sequencing batch reactor (SBR) received filtered primary effluent from a pilot WWTP treating municipal wastewater. Primary effluent was filtered through a nylon bag with 100 µm pore size (Infiltec GmbH, type BNM-100-01-SR) to remove part of the influent unbiodegradable particulate fraction. According to Johnson et al. (2008) and Menniti et al. (2012) screening at 250 µm pore size removes 70-100% of the influent unbiodegradable particulate fraction ($X_{U,Inf}$) and the screening removes selectively $X_{U,Inf}$ (Comeau et al. 2010, Labelle et al. 2015).

Activated sludge from synthetic wastewater

Activated sludge fed with synthetic WW was cultivated according to Habermacher et al. (2015). The experimental system used for the cultivation of this sludge consisted of an SBR coupled to a side stream reactor (SSR) for sludge reduction. Waste activated sludge was collected during four days at ambient temperature and then stored for four days at 4 °C before the startup of the digestion experiment. The collected sludge had an average SRT of 37 days (details: Table 3.1 and Habermacher et al. (2015)). The long SRT of the sludge was supposed to give a lower EPS content as compared to the activated sludge from real wastewater (SRT of five days).

Batch biomass

Batch biomass was grown in a liquid culture media. We hypothesized that this biomass would be characterized by no or only little aggregation (mainly suspended cells). Since aggregation is driven by EPS, a low aggregation means low abundance of EPS. This could influence the degradability since EPS has a different degradability than microbial cells (Laspidou and Rittmann 2002a, Ramdani et al. 2012b). However, microscopic observations indicated the formation of flocs. The batch biomass was harvested after 40 days of incubation for the digestion experiments.

Sludge digestion experiments

The three different sludges were washed prior to the digestion tests. For this, the sludges were centrifuged, the supernatant was discarded and the sludge pellets were retained. The sludge pellets were re-suspended in secondary effluent of a pilot scale WWTP treating municipal

wastewater. Since the volume of the removed supernatant was not equal to the added volume of secondary effluent, the mixed liquor VSS concentration (MLVSS) was modified by the washing step. The re-suspension in the same bulk liquid provided a similar degradation environment for the different solids. Each re-suspended sludge was divided into two portions of six liters. One portion was used for digestion under full aeration and the other for intermittent aeration. The sludges were continuously stirred during digestion. In the first two weeks, the intermittent aeration of the sludges from real wastewater and from the batch biomass was set to alternate from six hours aeration to six hours non-aeration. After two weeks, the oxygen input was lowered by reducing the aeration time (one hour per day) and by adding floating plastic balls to decrease the surface oxygenation rate. The intermittent aeration of the activated sludge from synthetic wastewater was set to alternate from six hours aeration to six hours non-aeration during the first week of operation. For this reactor, the oxygen input was reduced already after one week by a reduced aeration time (one hour per day) and by the addition of floating plastic balls. The volume of water lost by evaporation was compensated by demineralized water. Temperature and pH were manually measured with a maximum frequency of once per day. Minimum and maximum measured temperatures were 16 and 26.3 °C, respectively. Minimum and maximum pH were 5.8 and 8.6, however pH was adjusted to 7.5 after measurements, with HCl or NaOH.

Analyses

Volatile suspended solids (VSS) were measured in duplicates or triplicates according to standard methods (APHA et al. 2005). Replicate VSS values were averaged for display and consecutive calculations. Oxygen concentration, pH and temperature were measured with portable devices (WTW 340i).

Bound extracellular polymeric substances

Bound EPS were extracted with a combination of methods; sonication, the non-ionic detergent Tween and the cation chelator EDTA were used in sequence according to Ras et al. (2008). This sequence of extractions was repeated twice. The extracts were analyzed for proteins by the BCA method (Smith et al. 1985) using BSA as standard. The carbohydrates were analyzed by the Anthrone method (Dreywood 1946) using glucose as standard. A commercial kit (Sigma, BCA1) was used for protein quantification. A sample volume of 10 µl (instead of 50 µl) was

added to 400 µl of reagent to overcome EDTA interferences during protein quantification. The activity of G6P-DH was measured to check cell lysis (Lessie and Wyk 1972). No cell lysis was detected compared to the positive controls which contained purified G6P-DH enzymes in the different extraction buffers. Protein and carbohydrate concentrations of all extracts were determined in duplicates. To express the content of bound EPS, measured EPS concentrations (mg EPS L^{-1}) were normalized by the initial VSS concentration of the sludge. The overall bound EPS was considered as sum of extracted proteins and carbohydrates.

Adenosine tri-phosphate

Total Adenosine tri-phosphate (ATP) concentrations were measured to evaluate the decay of cellular biomass. Soluble ATP concentrations were measured from sludge filtrates (0.45 µm glass-fiber filters). BacTiter-Glo (commercial kit, Promega Corporation) was used for measuring ATP concentrations. Sludge samples were diluted 50 times in deionized water, heterogeneous sludge samples were homogenized prior to dilution, 100 µl sample were mixed with 100 µl reagent, the assay temperature was kept constant at 30 °C and initial incubation was carried out under intense mixing (vortex) for 10 seconds. Total ATP concentration was measured in duplicates or triplicates. Average values were calculated and used in consecutive calculations and display. Intracellular ATP concentration was calculated as the difference between the total and soluble ATP concentrations. Intracellular ATP concentration was normalized by the VSS concentration.

Statistical model for VSS degradability

Statistical tests were performed to evaluate the biodegradation of VSS submitted to long-term batch digestion. An exponential degradation model $\ln(VSS)=-b_{VSS}*t + \ln(VSS_0)$ was fitted to the VSS measurements, assuming normal and independent distributed errors. F-Test was used to compare the estimated rate b_{VSS} against the null hypothesis $b_{VSS}=0$. Only VSS data recorded after 30 days were considered for this statistical analysis because we assumed that most of the active biomass was degraded after 30 days as reported in literature (Ramdani et al. 2012b). Nevertheless, an identical statistical analysis was performed on VSS data after 100 days of digestion. Statistical test after 100 days was chosen to guarantee absence of important amounts of active biomass.

Results

Degradability of activated sludge during very long digestion time

The change in the residual VSS concentrations during continuous or intermittent aeration is shown for the three different sludges over 400 days of digestion (Fig. 3.1). The residual VSS decreased rapidly under all conditions during the first 30 days of digestion (Fig. 3.1). Around 50% to 80% of total sludge degradation was indeed observed during this period. The most rapid and extended decrease was noticed for activated sludge cultivated with real wastewater (residual VSS of 36% measured after 30d under fully aerated conditions, Fig. 3.1). Lower decrease in the sludge concentration was observed for the two other sludges (residual VSS concentration of 51 to 53% under fully aerated conditions after 30 days of digestion). Simultaneously to the VSS degradation, the intracellular ATP content also decreased rapidly during the first 30 days of digestion as shown for activated sludge from real wastewater and batch biomass (Fig. 3.1 – third row). From day 30 the VSS concentration then steadily decreased for all conditions tested except for the batch biomass under condition of intermittent aeration (Fig. 3.1, Table 3.2). After 100 days of digestion, the decrease of VSS continued for three conditions out of six considering a significance level of 5% (Table 3.2). Such VSS degradation after 100 days was observed for the activated sludge from synthetic wastewater under both aeration conditions and for the batch biomass under fully aerated condition only.

Statistical analysis was conducted to assess the degradation rate coefficients of VSS after an extended period of digestion (i.e. after 30 days or after 100 days) (Table 3.2). VSS degradation was characterized by first order rate coefficients of 1 to $2.7 \cdot 10^{-3}$ d^{-1} (Table 3.2) considering only the sludges with statistical significant degradation from day 100 to 400. In comparison, Ramdani et al. (2012a) and Spérandio et al. (2013) measured a higher rate of $7 – 7.5 \cdot 10^{-3}$ d^{-1} (Table 3.2). The values from literature are thus 2.6 to 7 times higher than in the present experiment, considering only the sludges with statistical significant degradation from day 100 to 400.

Table 3.2 : Degradation rate coefficient for volatile suspended solids (b_{VSS}), for digestion time larger than 30 days (upper row) and larger than 100 days (bottom row). In brackets are the P-values from F-test comparing the fitted exponential model for VSS against a constant model. Values in bold are statistically significant at a level of 5%.

	Activated sludge from real wastewater		Activated sludge from synthetic wastewater		Batch biomass		References (20 °C)
	full aeration	intermittent aeration	full aeration	intermittent aeration	full aeration	intermittent aeration	(Ramdani et al., 2012a), (Spérandio et al., 2013)
b_{VSS} (d^{-1}), time 30-400 days (p-value)	**$1.5*10^{-3}$** (0.007)	**$1.6*10^{-3}$** (0.017)	**$1.7*10^{-3}$** (<0.001)	**$3*10^{-3}$** (<0.001)	**$1.3*10^{-3}$** (0.005)	$0.9*10^{-3}$ (0.210)	
b_{VSS} (d^{-1}), time 100-400 days (p-value)	$1.1*10^{-3}$ (0.061)	$0.7*10^{-3}$ (0.151)	**$1.6*10^{-3}$** (0.001)	**$2.7*10^{-3}$** (<0.001)	**$1.0*10^{-3}$** (0.044)	$0.1*10^{-3}$ (0.891)	$7.0\text{-}7.5*10^{-3}$

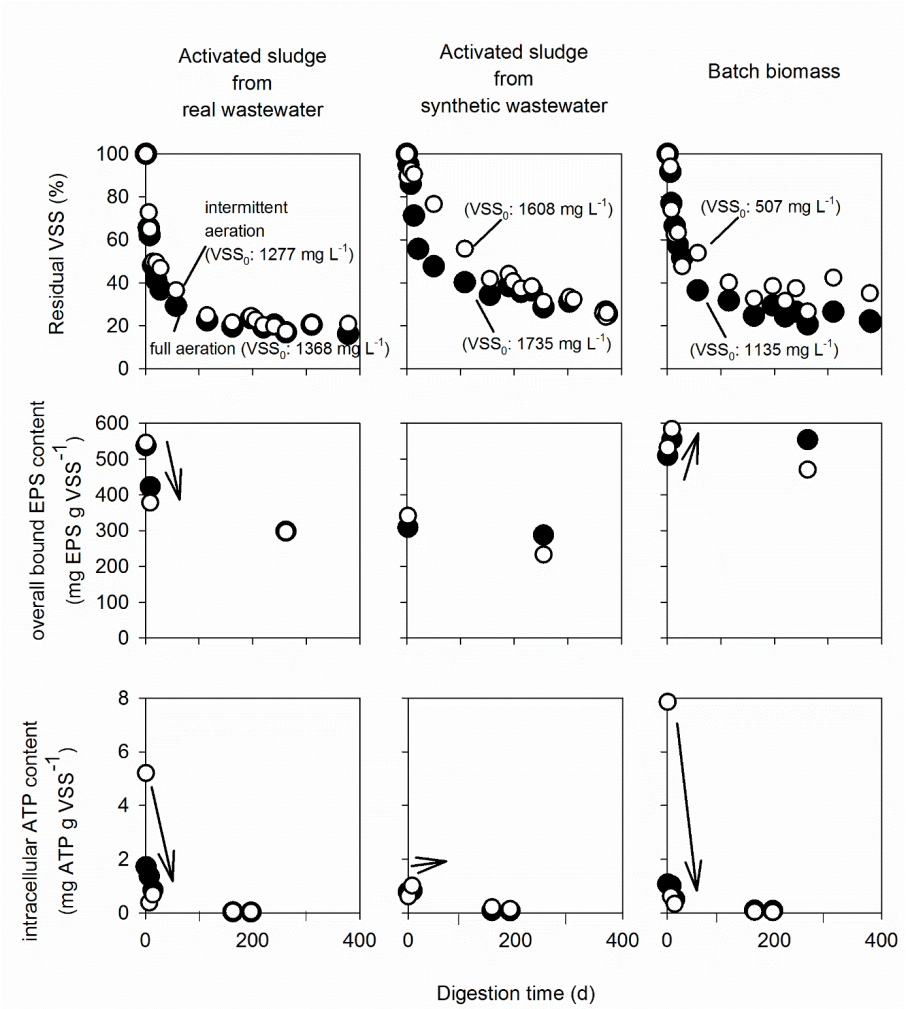


Fig. 3.1 Residual concentrations of volatile suspended solids (VSS) as percentage of initial VSS concentration over digestion period (first row) and content of bound extracellular polymeric substances (EPS) and intracellular adenosine tri-phosphate (ATP) in sludge (second and third row, respectively). In the first row, VSS_0 indicates the initial VSS concentration at the beginning of the digestion experiment. Arrows were put to indicate the initial dynamics, which are otherwise difficult to recognize. The standard deviation of VSS replicates was 4% of the mean value, in average, for the intracellular ATP this value was at 17%. The method standard deviation for overall bound EPS is 9% of the mean value.

Degradability of overall bound EPS and pools of bound EPS

A significant degradation of the overall bound EPS was monitored (Fig. 3.2). The concentrations of overall bound EPS decreased from 700 to 70 mg EPS L^{-1} for activated sludge fed with real wastewater (Fig. 3.2, Table S 3.1, Table S 3.2). The concentration of overall bound EPS also significantly decreased for the other sludges. The content of overall bound EPS (mg EPS g VSS^{-1}) decreased from 550 to 300 mg EPS g VSS^{-1} in the activated sludge from real wastewater (Fig. 3.1). The overall bound EPS content especially decreased during the first week of digestion for activated sludge from real wastewater. The value of overall bound EPS content decreased for more than 20% under both aeration conditions (Fig. 3.1). The value of bound EPS content decreased less rapidly for this sludge between day 8 to day 262, (about 25%). In comparison, the content of overall bound EPS decreased to a lower extent for the other two sludges, under both aeration conditions.

Bound protein and bound carbohydrate contents showed different trends during digestion. For the two activated sludges, the contents of bound protein decreased while the bound carbohydrate contents either remained stable or slightly increased (Fig. 3.3, first and second row). An opposite trend was observed for the batch biomass. The content in bound proteins decreased significantly for proteins extracted with Tween and to a slightly lower extent for the proteins extracted by sonication (Fig. 3.3). The content in bound proteins extracted with EDTA increased over digestion time (Fig. 3.3). Bound carbohydrates were present in smaller amounts than bound proteins. But the higher reduction of bound carbohydrates was also observed for the ones extracted with Tween (Table S 3.1 – S 3.6).

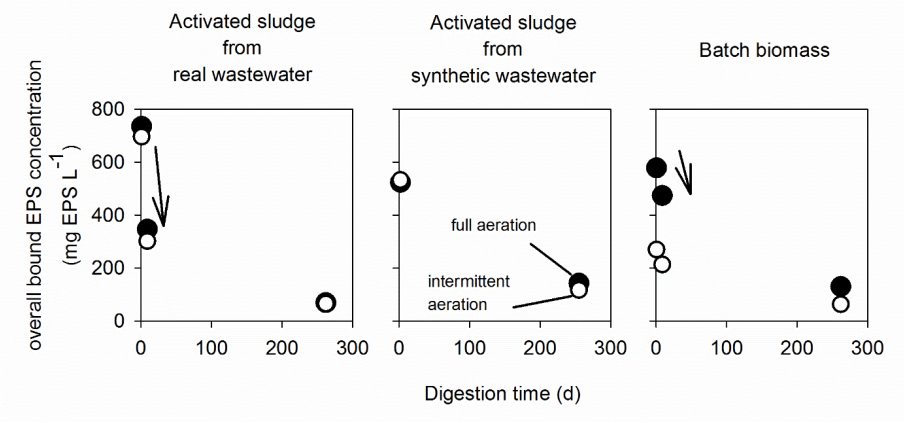


Fig. 3.2 Change in the overall bound EPS concentrations. Arrows were put to indicate the dynamics of the change in overall bound EPS.

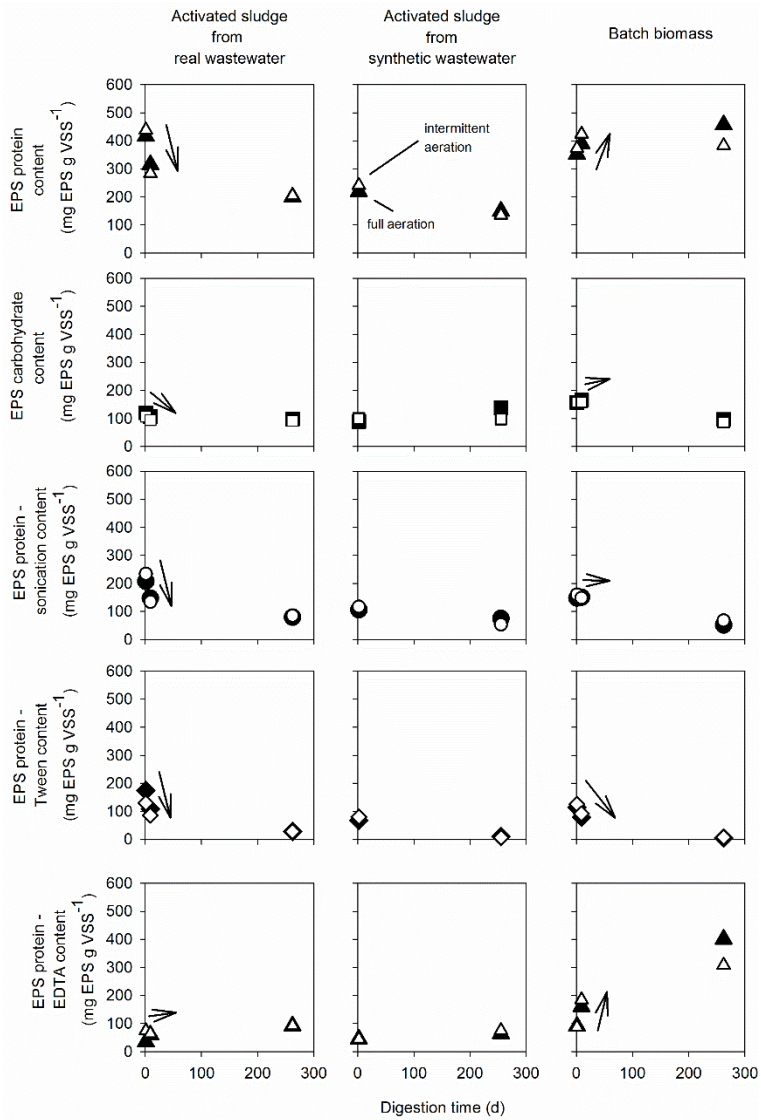


Fig. 3.3 Bound extracellular polymeric substances (EPS) proteins and carbohydrates content in sludges under full aeration digestion (plain symbols) and intermittent aeration digestion (void symbols) (first two rows). Bound EPS proteins extractible by sonication and Tween are plotted in the third and fourth row, bound EPS proteins extractible by EDTA are plotted in the bottom row. Arrows were put to indicate the initial dynamics, which are otherwise difficult to recognize.

Discussion

The unbiodegradable particulate fraction from real activated sludge is biodegradable

The main finding of our study is that the unbiodegradable particulate fraction of sludge fed with real municipal wastewater is biodegradable in absence of large amounts of active biomass. This was proven by the steadily VSS decrease observed after 30 days respectively 100 days of batch digestion (Fig. 3.1, Table 3.1). Within the first 30 days of digestion the extended VSS degradation results from the degradation of active biomass. This was indicated in our study by the strong depletion of intracellular ATP observed during this period for activated sludge from real wastewater. Intracellular ATP was demonstrated to be a good quantification method for cellular biomass in Hammes et al. (2010). The measured contents of ATP at the beginning of digestion were similar to the ones of Jørgensen et al. (1992) (3 mg ATP g VSS^{-1}). Ramdani et al. (2012b) also reported that 99% of the active biomass decayed after 21 days of aerobic batch digestion based on measurements of oxygen uptake rates. Therefore, we can assume that the amount of active biomass after 30 days of digestion is almost nil for the activated sludge from real wastewater and that the subsequent VSS degradation results from the degradation of another sludge fraction. The degradability of VSS after 30 days of digestion is thought to be explained more by the degradation of the endogenous residues than by degradation of the influent unbiodegradable particulate fraction. The activated sludge from real wastewater was produced on filtered primary effluent. The filtration at 100 um pore size removed presumably most of the influent unbiodegradable particulate fraction. That assumption is based on literature reports: i) $X_{U,Inf}$ removal efficiencies of 70-100 % by screens with pore size around 250 µm (Johnson et al. 2008, Menniti et al. 2010) and ii) selective removal of $X_{U,Inf}$ with such screens (Comeau et al. 2010, Labelle et al. 2015). The degradability of endogenous residues was already reported by Ramdani et al. (2012a) under similar conditions but with a sludge fed with synthetic wastewater. Our work demonstrates that the unbiodegradable particulate fraction degrades also for activated sludge fed with real wastewater.

The fact that the unbiodegradable particulate fraction is biodegradable at ambient temperature has practical implications for several processes. The biodegradability of the unbiodegradable particulate fraction implies that sludge minimization at very long SRT is possible. So far, this

was shown for MBRs or for systems based on anaerobic digestion. But such processes may not be relevant for decentralized wastewater treatment.

The influence of sludge origin and degradation conditions on the degradability of the unbiodegradable particulate fraction

The degradability of the unbiodegradable particulate fraction was confirmed for sludges with different histories (Fig. 3.1, Table 3.2). The actual variation in the degradation rate coefficient, b_{VSS}, for the three different sludges is small. A larger and more important difference is observed between the measured values in this experiment and previously published values.

Under full aeration, the sludges were characterized by b_{XU} of 1.3 - 1.7 · 10^{-3} d^{-1} (period 30-400 days) respectively 1-1.6 · 10^{-3} d^{-1} (period 100-400 days) (Table 3.1). These rates show some variation, but in comparison to previously published values of 7-7.5 · 10^{-3} d^{-1} (Ramdani et al. 2012a, Spérandio et al. 2013) the variability within the three tested sludges is small. The degradability of the unbiodegradable particulate fraction is estimated primary on the full aeration sludges. The intermittent aeration digestions also provide useful information but the interpretation has to be done more carefully in two cases. First, the residual VSS of the batch biomass did not significantly decrease under intermittent aeration condition after 30 days of digestion. The decrease of VSS in that particular case (batch biomass under intermittent aeration) was probably not measurable due to a low precision of VSS measurements. The precision of VSS measurements was lower than for the other five digestion reactors (indicated by the scattered values of residual VSS after 30 days of digestion (Fig. 3.1)). The precision of residual VSS values decreased over the digestion period for all digesting sludges. Presumably this was due to decreasing sludge concentrations. Indeed, the batch biomass under intermittent aeration had a clearly lower solids concentration already at the beginning of digestion (500 mg VSS L^{-1}) as compared to the other five digestion reactors (1100-1700 mg VSS L^{-1}). Therefore the precision of residual VSS for this sludge was especially low and thus the VSS statistic disturbed. Second, the VSS degradation of activated sludge from synthetic wastewater under intermittent aeration was presumably still affected by significant decrease of active biomass, after 30 days and 100 days of digestion. This is indicated by the corresponding VSS dynamic (Fig. 3.1) in combination with lower oxygen concentrations during non-aerated periods (Fig. S

3.1). The low oxygen concentration in that reactor only can explain a slow decay rate of active biomass (Habermacher et al. 2015) in case of the activated sludge from synthetic wastewater.

The degradation rates of the unbiodegradable particulate fraction (b_{XU}) with statistical significance were six to ten times smaller than those reported from model fitting by Ramdani et al. (2012a) or by Spérandio et al. (2013). One hypothesis is that the value of b_{XU} decreases over the digestion time. Such phenomenon might not be observable in previous experiments because limited digestion times were applied (150 days in Ramdani et al. (2012a) or 120 days in Martínez-García et al. (2016)). If b_{XU} decreases over digestion time, simulations considering close to zero excess sludge production, such as in Fall et al. (2014), need to be adjusted to b_{XU} values as low as determined in this study. Other factors might also explain the small values of b_{XU} measured in our study. Temperature may explain only part of this difference. Ramdani et al. (2012a) had determined a temperature correction factor for b_{XU}. Even if b_{XU} of Ramdani et al. (2012a) and Spérandio et al. (2013) are corrected towards the lowest temperature that was measured in our experiment (i.e., 16 °C), the b_{XU} of our activated sludges from real wastewater would still remain five to eight times smaller. Martinez-Garcia et al. (2016) suggested that low pH can increase $b_{XU,E}$. Our results do not support the influence of pH on b_{XU}. The pH value of the activated sludge from synthetic wastewater varied between 6 and 7 under full aeration and remained stable at around 8 under intermittent aeration between day 150 and 400 (Fig. S 3.2). Despite these different pH values, the VSS dynamic of both batch reactors was identical between day 150 and 400 for that sludge (Fig. 3.1). Further, Ramdani et al. (2012) operated a membrane digestion unit at a pH of 7-7.3, thus at similar pH range as our reactors, but they observed a high $b_{XU,E}$ of 7 10^{-3} d^{-1} (i.e. 5-10 times higher than our observations). Thus, it can be assumed that pH in the range of 6 to 8 is not a determinant factor for the degradation of the unbiodegradable particulate fraction. Other factors that could influence $b_{XU,E}$ are the shear force (Spérandio et al. (2013)) , the abundance of active biomass or the composition of the digestion liquid.

Extracellular polymeric substances (EPS) are also biodegradable

Our second main finding is that overall bound EPS are biodegradable as demonstrated by the decrease in the concentrations of overall bound EPS (Fig. 3.2). Such mechanism was observed for sludge of different origins, i.e., with different initial composition in terms of overall bound

EPS and specific EPS pools. The biodegradability of overall bound EPS was previously observed in several studies (Habermacher et al. 2015, Hsieh et al. 1994, Menniti and Morgenroth 2010, Ramdani et al. 2012b). However, these studies were limited to rather short digestion times (up to 20 days) and only one type of sludge was tested. Our results demonstrate that noticeable degradation of bound EPS still occurs at very long SRT (after 30d of digestion) (Fig. 3.2). Thus, bound EPS are not less biodegradable than other sludge fractions and do not accumulate in the unbiodegradable particulate fraction (Fig. 3.1).

The dynamic of overall bound EPS degradation follows the degradation dynamic of sludge fractions, i.e. active biomass and unbiodegradable particulates. The rapid initial decrease of overall bound EPS content, observed for the activated sludge from real wastewater, confirms that fairly biodegradable EPS are initially associated with active biomass. Thereafter, the residual bound EPS degrades at a similar rate as VSS as indicated by the constant bound EPS content of the sludge (Fig. 3.1). Over long term, the residual bound EPS are thus associated to the unbiodegradable particulate fraction. This is in accordance with the conceptual model proposed by Ramdani et al. (2012b); bound EPS initially associated with the active biomass, and then associated with the endogenous residues after 30 days of digestion. Thus, activated sludge models do not need to integrate overall bound EPS as an additional state variable for accurate prediction of sludge production at very long SRTs.

Our results also demonstrate that the different EPS pools degrade at different rates. However, this had a limited influence on the overall bound EPS degradation observed in our study. Indeed, the initial compositions of the sludges in terms of bound EPS were rather similar (Fig. 3.3). The high protein contents decreased over the digestion period for the two activated sludges. On the contrary, the low carbohydrate contents remained constant or increased. Bound proteins were thus characterized by a higher biodegradability than bound carbohydrates with exception of the batch biomass (discussed below). This confirms previous findings of Ramdani et al. (2012b) and Habermacher et al. (2015). Also, different extraction methods can extract specific types of bound EPS compounds (Park and Novak 2007, Park et al. 2008, Ras et al. 2011). Tween can extract hydrophobic EPS and EDTA can extract cation bound EPS (Ras et al. 2008). The initial content in cation bound EPS proteins was low but increased over the digestion time. The opposite trend was observed for hydrophobic bound EPS proteins. This was consistent for the

three different sludges. If cation bound EPS proteins are biodegradable at a slower rate than other bound EPS pools, then a sludge with a high initial content in cation bound EPS proteins would have a slow degradability. This might explain why the bound EPS protein content of the batch biomass did not decrease over digestion time, in contrast to the two activated sludges. However, the amount of cation bound EPS proteins in the initial sludge composition was limited (30-90 mg g VSS^{-1}). Thus, the degradation of the batch biomass can only be affected to a limited extent by its initial content in cation bound EPS.

Overall, our results on the biodegradability of the different EPS pools have several practical implications. Technologies operating at high solubilization rate could target the abundant bound EPS proteins of fresh activated sludge, e.g. with protease (Wawrzynczyk et al. 2008). Instead, the extent of sludge degradation could be increased with a chemical pretreatment such as EDTA that targets the resistant cation bound EPS (Nguyen et al. 2014).

Conclusions

- The unbiodegradable particulate fraction of sludge fed with real municipal wastewater is biodegradable in absence of large amounts of active biomass. The biodegradation was shown at ambient temperature and under oxic conditions. Volatile suspended solids (VSS) indeed steadily decreased after 30 days and after 100 days of digestion for this sludge. Therefore, an extended sludge minimization can be achieved by extending the SRT of oxic digesters. However degradation rates of the unbiodegradable particulate fraction were very low ($0.7\text{-}1.6 \cdot 10^{-3}$ d^{-1}).
- The bound EPS remain biodegradable over very long digestion time (up to 260 days). Stable ratios of bound EPS to VSS were indeed monitored over the entire digestion phase. Therefore the bound EPS do not accumulate as an inert component in the unbiodegradable particulate fraction.
- The active biomass affects greatly the biodegradation of the bound EPS. One part of the overall bound EPS is associated to active biomass and is fairly degradable. Another factor that affects EPS biodegradability is the composition of overall bound EPS. Hydrophobic bound EPS proteins degraded quickly in comparison to cation bound EPS proteins. Thus, for sludges that have very different overall bound EPS composition, the degradability of overall bound EPS can be affected.

Supplementary Information

A) Measurements of bound EPS compounds during digestion, concentrations in mass per volume

Table S 3.1 : Activated sludge from real wastewater, full aeration – concentrations in mass per volume of different compounds (mg L^{-1}). "- sonication", "- Tween" and "- EDTA" indicate by which method the proteins or carbohydrates were extracted.

	1 day digested	9 days digested	262 days digested
overall bound EPS	735	346	69
bound EPS proteins	570	259	46
bound EPS carbohydrates	165	88	23
bound EPS proteins - sonication	284	121	19
bound EPS proteins - Tween	239	89	7
bound EPS proteins - EDTA	48	49	21
bound EPS carbohydrates - sonication	61	38	13
bound EPS carbohydrates - Tween	50	28	3
bound EPS carbohydrates - EDTA	55	22	7

Table S 3.2 : Activated sludge from real wastewater, intermittent aeration – concentrations in mass per volume of different compounds (mg L^{-1}). "- sonication", "- Tween" and "- EDTA" indicate by which method the proteins or carbohydrates were extracted.

	1 day digested	9 days digested	262 days digested
overall bound EPS	696	302	66
bound EPS proteins	560	227	45
bound EPS carbohydrates	136	75	20
bound EPS proteins - sonication	299	107	19
bound EPS proteins - Tween	166	68	6
bound EPS proteins - EDTA	95	51	20
bound EPS carbohydrates - sonication	57	31	11
bound EPS carbohydrates - Tween	30	22	3
bound EPS carbohydrates - EDTA	49	22	6

Table S 3.3 : Activated sludge from synthetic wastewater, full aeration – concentrations in mass per volume of different compounds (mg L^{-1}). "- sonication", "- Tween" and "- EDTA" indicate by which method the proteins or carbohydrates were extracted.

	2 day digested	255 days digested
overall bound EPS	524	143
bound EPS proteins	372	74
bound EPS carbohydrates	152	69
bound EPS proteins - sonication	180	37
bound EPS proteins - Tween	116	5
bound EPS proteins - EDTA	76	32
bound EPS carbohydrates -sonication	58	47
bound EPS carbohydrates -Tween	36	4
bound EPS carbohydrates -EDTA	58	18

Table S 3.4 : Activated sludge from synthetic wastewater, intermittent aeration – concentrations in mass per volume of different compounds (mg L^{-1}). "- sonication", "- Tween" and "- EDTA" indicate by which method the proteins or carbohydrates were extracted.

	2 day digested	255 days digested
overall bound EPS	533	117
bound EPS proteins	376	68
bound EPS carbohydrates	156	49
bound EPS proteins - sonication	182	27
bound EPS proteins - Tween	125	3
bound EPS proteins - EDTA	69	38
bound EPS carbohydrates -sonication	64	26
bound EPS carbohydrates -Tween	38	4
bound EPS carbohydrates -EDTA	54	19

Table S 3.5 : Batch biomass, full aeration – concentrations in mass per volume of different compounds (mg L^{-1}). "- sonication", "- Tween" and "- EDTA" indicate by which method the proteins or carbohydrates were extracted.

	1 day digested	9 days digested	262 days digested
overall bound EPS	578	474	130
bound EPS proteins	400	333	107
bound EPS carbohydrates	178	142	23
bound EPS proteins - sonication	168	129	12
bound EPS proteins - Tween	130	67	1
bound EPS proteins - EDTA	103	137	94
bound EPS carbohydrates - sonication	73	75	7
bound EPS carbohydrates - Tween	75	29	2
bound EPS carbohydrates - EDTA	31	38	14

Table S 3.6 : Batch biomass, intermittent aeration – concentrations in mass per volume of different compounds (mg L^{-1}). "- sonication", "- Tween" and "- EDTA" indicate by which method the proteins or carbohydrates were extracted.

	1 day digested	9 days digested	262 days digested
overall bound EPS	270	213	64
bound EPS proteins	189	155	52
bound EPS carbohydrates	80	58	12
bound EPS proteins - sonication	81	54	9
bound EPS proteins - Tween	64	33	1
bound EPS proteins - EDTA	45	68	42
bound EPS carbohydrates - sonication	31	29	4
bound EPS carbohydrates - Tween	21	13	1
bound EPS carbohydrates - EDTA	28	16	6

B) Measurements of pH and oxygen concentrations in the batch reactors.

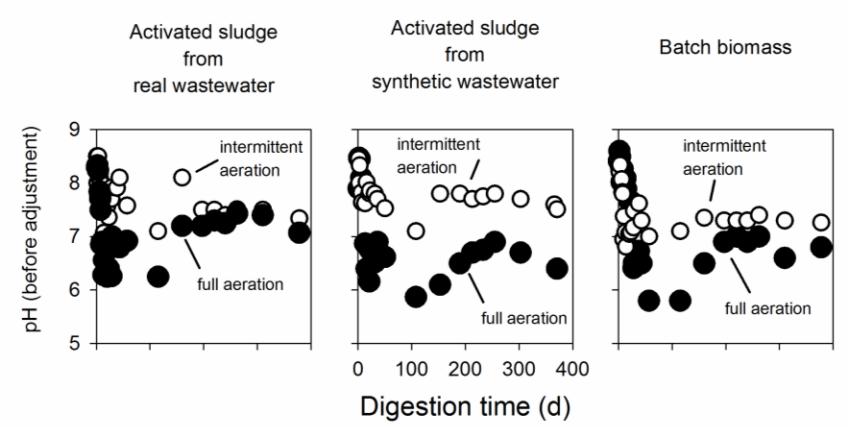


Fig. S 3.1 pH measurements in the batch reactors before the adjustments of the pH. The pH adjustments were done with NaOH or HCl to a pH of 7.5 (after the pH measurement).

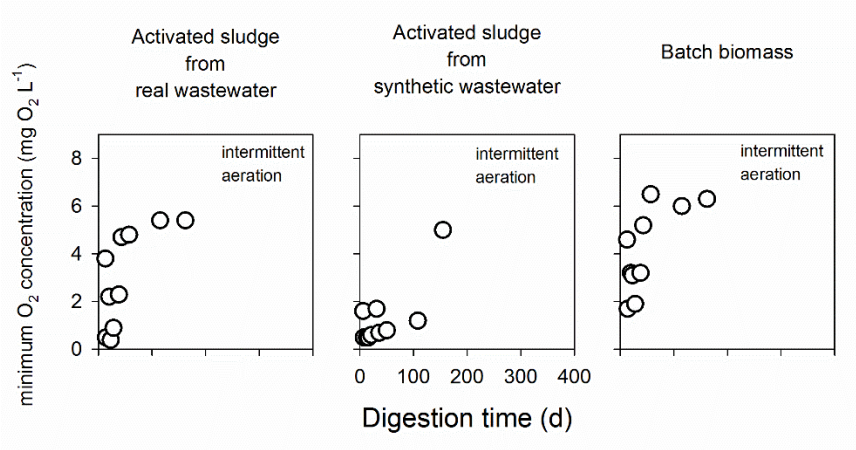


Fig. S 3.2 Minimum oxygen concentration in the intermittent aerated reactors, measured in the periods when the aeration was turned off.

Chapter 4

Modeling biodegradation of endogenous residues in the activated sludge – side-stream reactor treatment system: estimation and sensitivity of the endogenous residues decay rates

Habermacher, J., Menniti, A., Boltz, J.P., Rieger, L., Johnson, B.R., Daigger, G.T., Derlon, N., Morgenroth, E., (2017). Modeling biodegradation of endogenous residues in the activated sludge – side-stream reactor treatment system: estimation and sensitivity of the endogenous residues decay rates. In preparation.

Highlights

- We studied the biodegradation of endogenous residues in AS-SSR systems by modeling
- Endogenous residue decay rates ($b_{XU,E}$) estimated for 12 datasets from literature
- All estimated $b_{XU,E}$ values were positive and associated with a large distribution
- Localisation of sludge wastage in AS-SSR affects sludge yield and $b_{XU,E}$ estimation
- $b_{XU,E}$ values are sensitive to the influent iron concentration and feeding mode

Abstract

Recent studies suggest that endogenous decay residues ($X_{U,E}$) are biodegradable in activated sludge systems operated at very long SRT of 100 - 250 days. Activated sludge system equipped with side-stream reactors (AS-SSRs) allow for long SRTs and specifically aim at improving solid degradation. However, it is not clear if $X_{U,E}$ degradation occurs in AS-SSR and to what extent this mechanism contribute to the overall solids degradation. The current paper evaluated twelve published data sets obtained from AS-SSR systems using mathematical modeling to estimate the endogenous residues decay rate ($b_{XU,E}$). Estimated $b_{XU,E}$ values were larger than zero (median = 0.014 d^{-1}) which indicates endogenous residues biodegradation occurs in the evaluated system. The estimated $b_{XU,E}$ was shown to depend on the location of sludge wastage in the AS-SSR system. In addition, variation of $b_{XU,E}$ was identified due to bulk-phase water characteristic and process operational condition.

Keywords

endogenous residues, decay, active biomass, excess sludge reduction, side stream reactor.

Nomenclature

(notation according to Corominas et al. (2010))

$X_{U,E}$ (kg COD m^{-3})	Endogenous residues
X_{OHO} (kg COD m^{-3})	Ordinary heterotrophic organisms
XC_B (kg COD m^{-3})	Slowly biodegradable organic substrate
$X_{COD,Inf}$ (kg COD m^{-3})	Total particulate COD in the influent wastewater
$b_{XU,E}$ (d^{-1})	Decay rate of endogenous residues
$b_{OHO,endoresp}$ (d^{-1})	Decay rate of ordinary heterotrophic organisms in the endogenous respiration process (e.g. ASM3)
$b_{OHO,lys}$ (d^{-1})	Decay rate of ordinary heterotrophic organisms in the lysis process (e.g. ASM2d)
$f_{XU,E\_Bio,endoresp}$ (mg CODx mg $CODx^{-1}$)	Fraction of active biomass converted to endogenous residues in the endogenous respiration process (e.g. ASM3) using the endogenous respiration model
$f_{XU,E\_Bio,lys}$ (mg CODx mg $CODx^{-1}$)	Fraction of active biomass converted to endogenous residues in the lysis process (e.g. ASM2d), using the death-regeneration model
$f_{XU,Inf}$ (-)	Fraction of particulate unbiodegradable organics in the influent wastewater COD
$f_{SU,Inf}$ (-)	Fraction of soluble unbiodegradable organics in the influent wastewater COD
$i_{COD,XOHO}$ (kg CODx kg VSS^{-1})	COD to VSS conversion factor for ordinary heterotrophic organisms
$i_{COD,XU,E}$ (kg CODx kg VSS^{-1})	COD to VSS conversion factor for endogenous residues

Introduction

The handling and disposal of sludge is amongst the most expensive operational facets of municipal water resource recovery (MWRR). Sludge reduction offers viable means for significantly reducing associated operational expenses. Sludge reduction can be achieved through extended degradation of non-readily biodegradable materials at very long solid retention time (SRT) of more than 100 days (Menniti et al. 2012, Spérandio et al. 2013). Reduction of sludge requires an improved understanding of different sludge fractions relative biodegradability.

Major components contributing to the sludge production are the active biomass (e.g., ordinary heterotrophic organisms (X_{OHO})), endogenous residues ($X_{U,E}$), and inert organic particulates from the influent ($X_{U,Inf}$). The fraction of $X_{U,E}$ in activated sludge increases with an increasing SRT due to the decay of active biomass (Fall et al. 2014, Labelle et al. 2015, Paul and Liu 2012). Existing activated sludge models (ASM) (Henze et al. 2000) which were developed based on activated sludge systems operating at low to moderate SRTs, generally less than 20 days, do not consider $X_{U,E}$ to be biodegradable. The accumulation of $X_{U,E}$ can hamper the performance of systems operated at long SRTs, as the high solid inventory can result in problems of settling (Paul and Liu 2012) or the aeration (Fall et al. 2014). However recent studies suggest that $X_{U,E}$ is biodegradable at ambient temperature (Menniti et al. 2012, Ramdani et al. 2012a, Spérandio et al. 2013) and thus application of very long SRTs might be interesting. The decay of $X_{U,E}$ potentially is a key mechanism for the reduction of excess sludge in activated sludge systems operating at very long SRT.

Several recent studies focused on improving the solubilization of solid materials by exposing the sludge to long SRT and alternating redox conditions in activated sludge - side-stream reactors (AS-SSRs) (Chon et al. 2011, Habermacher et al. 2015, Huang and Goel 2015, Huang et al. 2014, Menniti et al. 2012, Novak et al. 2007, Yagci et al. 2015). Sludge exposure to alternating redox conditions is achieved by sequentially recirculating sludge from the (aerobic zone of a) suspended growth bioreactor to the SSR and vice-versa (Johnson et al. 2008). The observed biomass yield (Y_{obs}) for an AS-SSR system is typically reduced by 38 to 87% when compared to systems that do not operate an SSR (Chon et al. 2011, Huang and Goel 2015,

Huang et al. 2014, Menniti et al. 2012, Novak et al. 2007, Yagci et al. 2015). While the reduced overall sludge production in AS-SSRs may be hypothesized to be due to the solubilization of $X_{U,E}$, the rate of $X_{U,E}$ solubilization in such systems remains unclear. A better understanding of the decay process of $X_{U,E}$ in AS-SSRs is thus required to better predict the Y_{obs} of such systems, and their resulting sludge reduction performances. Especially, it is key to understand to what extent the degradation of $X_{U,E}$ contributes to the reduction of the overall sludge production.

Only one study quantified $X_{U,E}$ degradation in AS-SSR system by a numerical model fitting approach (Menniti et al. 2012). A first order decay coefficient, $b_{XU,E}$, of 0.0235 d^{-1} was reported in this study. This value for $b_{XU,E}$ is up to two orders-of-magnitude greater than values reported for other systems having a long SRT: 0.0001 d^{-1} – 0.0040 d^{-1} in batch digestion (Habermacher et al. 2016, Martínez-García et al. 2016), 0.007 - 0.0075 d^{-1} in membrane bioreactors (MBRs) (Spérandio et al. 2013) and batch reactors operated under various aeration conditions (Ramdani et al. 2012a). Values reported for $b_{XU,E}$ in these alternative systems were estimated using different modeling approaches for the estimation of $b_{XU,E}$. It remains unclear whether the higher value of $b_{XU,E}$ for AS-SSR systems (0.0235 d^{-1}) can be compared to $b_{XU,E}$ values from other investigations that used different models.

A first overall goal of the present study was to better understand if $X_{U,E}$ degradation is a significant process in AS-SSRs. This was accomplished by estimating $b_{XU,E}$ values for a variety of AS-SSR systems reported in the literature to determine whether they were systematically greater than zero. The second overall goal was to assess the sensitivity of $b_{XU,E}$ estimated values depending on the approach used to parameter estimation. Estimating the range of $b_{XU,E}$ values for the several AS-SSR systems reported in the literature allows examination of whether $X_{U,E}$ degradation appeared to occur. Some of these studies investigated the potential impact of various operating conditions on sludge yield. Consequently, this can help to identify the impact of these factors on $b_{XU,E}$.

Estimation of $b_{XU,E}$ values is based on mathematical models, e.g. by matching the predicted Y_{obs} or solids concentrations to the corresponding experimental values (Ramdani et al. 2012a, Spérandio et al. 2013). Slow decay rates that result in the effect of $X_{U,E}$ degradation being measurable only with high SRTs and the same effect of $X_{U,E}$ degradation on model results could

also be observed by manipulating model parameters other than $b_{XU,E}$ (Spérandio et al. 2013) which creates a parameter identifiability problem. Experimental data from high SRT systems are needed, along with an approach that limits problems associated with parameter identifiability. In this study modeling studies were carried out using previously reported data obtained from AS-SSR systems including: Novak et al. (2007), Chon et al. (2011), Huang et al. (2014), and Yagci et al. (2015). All these studies reported Y_{obs} values of AS-SSR systems operated at long SRT, compared to conventional systems generally operating at relatively long but more conventional SRTs. These studies offered the potential to estimate the range of $b_{XU,E}$ values using uncertainty analysis, as the values of Y_{obs} for the AS-SSR systems are directly linked to the degradation of $X_{U,E}$ (Spérandio et al. 2013).

Different operating conditions were recently suggested to influence $b_{XU,E}$. High temperatures increase the value of $b_{XU,E}$ (Ramdani et al., 2012). The pH (Martínez-García et al., 2016) and oxygen concentration (Liu and Wang, 2015) were also suggested to affect $b_{XU,E}$. Two other important characteristics, SRT and influent iron concentration, are hypothesized to influence $b_{XU,E}$ but lack quantitative assessment to date. Active biomass concentration, and thus implicitly SRT, was suggested to influence $b_{XU,E}$ in Menniti et al. (2012), but no quantitative assessment was provided. Cations such as trivalent iron (Fe III) are thought to interact with extracellular polymeric substances (EPS) (Novak et al. 2003). Since EPS are part of $X_{U,E}$ (Habermacher et al. 2016) iron could influence the degradability of $X_{U,E}$, but quantitative assessment does not currently exist. Fortunately the data provided by the data sets from the literature allows the impact of SRT and influent iron concentrations (S_{Fe}) on $b_{XU,E}$ to be assessed.

The specific objectives of this study are (i) to identify the range of $b_{XU,E}$ values for endogenous residue ($X_{U,E}$) transformation in AS-SSR systems and to evaluate if the predicted $b_{XU,E}$ values are larger than zero (ii) to estimate $b_{XU,E}$ using different models (analytical or numerical) with different structures in order to understand if the predicted values of $b_{XU,E}$ are sensitive to the model structures and thus to understand generally how comparable $b_{XU,E}$ values coming from different sources are, and (iii) to correlate predicted $b_{XU,E}$ values to operating conditions in order to identify what factors could influence the degradation of $X_{U,E}$ in AS-SSR systems. First the sensitivity of Y_{obs} towards $b_{XU,E}$ was assessed in order to determine the suitability of the data available from the literature for estimate $b_{XU,E}$ values for various systems, and the uncertainty

of the resulting estimates. The range of $b_{XU,E}$ values were then estimated for AS-SSR systems from selected studies reported in the literature. The approach for $b_{XU,E}$ estimation was based on comparing Y_{obs} value for the AS-SSR system to the Y_{obs} value of the control system using a consistent model to estimate the value of $b_{XU,E}$. Such a comparison of the sludge reduction performance between the AS-SSR system and the control system is more robust than fitting models only to the Y_{obs} of the AS-SSR. A steady-state analytical model was used to model of Y_{obs} for the two systems. Finally the influence of choosing a more complex biokinetic model or a more complex reactor model in the approach for $b_{XU,E}$ estimation was assessed.

Materials and methods

Overall approach for estimating endogenous residues decay rates ($b_{XU,E}$)

Experimental data (Y_{obs}) from the literature were used in this study. These data were collected for systems with or without SSRs (e.g., control systems). Our approach to estimate $b_{XU,E}$ values is based on calculating the relative difference between the Y_{obs} of the control and AS-SSR systems. The Y_{obs} reduction predicted by models is then fitted to the Y_{obs} reduction determined using experimental data. Fitting the predicted and experimental difference of Y_{obs} values was accomplished by simply varying the $b_{XU,E}$ value to obtain the closest fit between measured and predicted values. The main assumption of this approach is that the variation of Y_{obs} between the control and AS-SSR systems that is not explained by active biomass degradation results exclusively from the degradation of $X_{U,E}$. The overall approach comprises the following four steps:

1. Experimental values of Y_{obs} were collected from the literature (Novak et al. (2007), Chon et al. (2011), Huang et al. (2014), Yagci et al. (2015)) for the control systems (conventional activated sludge system only) and for experimental systems (i.e., AS-SSR).
2. The relative reduction in Y_{obs} values resulting from the addition of a SSR was calculated according to eq. (4.1) which was presented by Paul and Liu (2012):

$$\Delta Y_{obs,relative} = \frac{Y_{obs,control} - Y_{obs,AS-SSR}}{Y_{obs,control}} \quad (4.1)$$

where $\Delta Y_{obs,relative}$ is the relative reduction of the observed yield, based on $Y_{obs,control}$ the observed yield of the control system (mg VSS mg COD^{-1}), and $Y_{obs,AS-SSR}$ the observed yield of the AS-SSR system (mg VSS mg COD^{-1}).

3. A model, as described below, was applied to predict the Y_{obs} of the control and the AS-SSR systems, and in turn to predict $\Delta Y_{obs,relative}$.
4. The values of the $b_{XU,E}$ were identified by fitting the relative reduction in Y_{obs} values predicted by the model to the experimental values.

Models

Three models were applied in this study: an analytical steady-state model (Model No. 1, Heterotrophs - CFSTR), a numerical model that does not consider the influence of system configuration (Model No. 2, ASM2d extended – CFSTR), and a numerical model that considers the influence of system configuration (Model No. 3, ASM2d extended – AS-SSR).

Model No. 1 Heterotrophs - CFSTR

The analytical steady-state model used here for $b_{XU,E}$ is from Spérandio et al. (2013), eq. (4.2).

$$Y_{obs} = \left(\left(1 - f_{XU,Inf} - f_{SU,Inf}\right) \cdot \left(\frac{Y_{OHO}}{\left(1 + b_{OHO} \cdot SRT\right)} \cdot \frac{1}{i_{COD,XOHO}} + \frac{Y_{OHO} \cdot b_{OHO} \cdot f_{XU,E\_Bio,endoresp} \cdot SRT}{\left(1 + b_{OHO} \cdot SRT\right) \cdot \left(1 + b_{XU,E} \cdot SRT\right)} \cdot \frac{1}{i_{COD,XU,E}} \right) + \frac{f_{XU,Inf}}{i_{COD,XU,Inf}} \right) \cdot \frac{1}{1 - f_{SU,Inf}} \quad (4.2)$$

Model No. 1 describes Y_{obs} for an ideal continuous flow stirred tank reactor (CFSTR), with sludge retention from a coupled liquid-solids separation unit, as a function of the operational parameter SRT and biokinetic model parameters for growth and decay of ordinary heterotrophic organisms. Model No. 1 uses the endogenous respiration concept for predicting sludge reduction. Default parameter values presented in Spérandio et al. (2013) were used when the model was applied for use in this manuscript: (1) growth yield of ordinary heterotrophic organisms (OHO), Y_{OHO} = 0.67 mg COD_X mg COD^{-1}; (2) decay rate of OHO, b_{OHO} = 0.24 d^{-1}; (3) fraction of active biomass converted to endogenous residues during endogenous respiration,

$f_{XU,E\_Bio,endoresp}$ = 0.20 mg CODx mg $CODx^{-1}$; (4) COD-to-VSS conversion factor for OHO, $i_{COD,XOHO}$ = 1.42 mg COD mg VSS^{-1}; (5) COD-to-VSS conversion factor for endogenous residues, $i_{COD,XU,E}$ = 1.55 mg CODx mg VSS^{-1}; (6) COD-to-VSS conversion factor for influent unbiodegradable particulate organic matter, $i_{COD,XU,Inf}$ = 1.55 mg CODx mg VSS^{-1}; (7) fraction of soluble unbiodegradable organics in influent wastewater COD, $f_{SU,Inf}$ = 0.05. Individual values of Y_{obs}, SRT, and $f_{XU,Inf}$ (fraction of particulate unbiodegradable organics in the influent wastewater COD) were taken from the reported studies. Values of $b_{XU,E}$ were varied in order to match the observed relative Y_{obs} reductions.

Y_{obs} is the net sludge production rate (including sludge accumulation rate inside of the system and transportation rate outside of the system) over the rate of COD removal from the wastewater that is treated. Y_{obs} is used in the units of mg VSS mg COD^{-1} throughout this study. In this study SRT refers to the overall SRT of an activated sludge system, considering the total sludge mass in a system divided by the total sludge mass leaving the system, including sludge wastage, effluent suspended solids and sludge sampling.

Model No. 2 and No. 3: Influence of model structure and parameter values on $b_{XU,E}$ estimations using ASM2d

The influence of model structure and parameter values on estimation of the range of $b_{XU,E}$ values was assessed to determine to what extent model structure and parameter values influence the range of predicted $b_{XU,E}$ values. The approach for $b_{XU,E}$ estimation (described in the first section of materials and methods) was repeated but using two more complex models in step 3 (Table 4.1, Table 4.2). Model No. 2 consists of a process model of ASM2d with extension for $X_{U,E}$ degradation and a reactor model of a CFSTR with sludge retention from a coupled liquid-solids separation unit. Model No. 3 consists of ASM2d with extension for $X_{U,E}$ degradation and explicit modeling of the actual reactor configuration in the AS-SSR with sludge recirculation. A more detailed description of model No. 3 is given in the supplementary information. The influence of using a simple or a more complex biokinetic model for $b_{XU,E}$ estimation was evaluated by comparing $b_{XU,E}$ estimations generated with model No. 1 and No. 2 (Table 4.1). The influence of considering a simplified or a more complex reactor model for $b_{XU,E}$ estimation was evaluated by comparing $b_{XU,E}$ estimations generated with models No. 2 and No. 3 (Table 4.1). For models No. 2 and No. 3 the default parameter values of ASM2d were used (Henze et

al. 2000) with the process of endogenous residues decay added (Table 4.2). A simple first-order kinetic model for predicting the degradation of $X_{U,E}$ to slowly biodegradable organic substrate (XC_B) was used because of the limited mechanistic understanding that would justify the use of a more complex model (e.g. saturation type kinetics). To model Y_{obs} with models No. 2 and No. 3 numerical simulations were run in the simulation platform SUMO® (Dynamita, Nyon, France). The duration of each simulation run was chosen in order to reach steady state, i.e., when Y_{obs} and solids concentrations did not change anymore with increasing simulation time. Experimental data from Chon et al. (2011) were used for the assessment of the model structure on $b_{XU,E}$ estimation (Table 4.1). The endogenous decay model is used in model No. 1. For models No. 2 and No. 3, the death-regeneration model was used as it is the most common approach. However, these two modeling approaches (endogenous decay vs death-regeneration) are equivalent with regard to sludge production prediction (Dold et al. (1980)). To compare the endogenous decay approached used in model No. 1 to models No. 2 and No. 3, the decay parameters of the endogenous decay processes of model No. 1, $b_{OHO,endoresp}$ and $f_{XU\_Bio,endoresp}$, were expressed to equivalent parameters for the death-regeneration processes of models No. 2 and No. 3, namely $b_{OHO,lys}{}^{*}$ and $f_{XU,E\_Bio,lys}{}^{*}$, according to eq. (4.3) and (4.4) as presented by Dold et al. (1980). The parameters $b_{OHO,lys}$* and $f_{XU\_Bio,lys}{}^{*}$ were used for a diagnostic comparison, but not for modeling.

$$b_{OHO,lys}{}^{*} = \frac{(1 - Y_{OHO,lys} \cdot f_{XU,E\_Bio,endoresp})}{(1 - Y_{OHO,lys})} \cdot b_{OHO,endoresp} \tag{4.3}$$

$$f_{XU\_Bio,lys}{}^{*} = \frac{(1 - Y_{OHO,lys})}{(1 - Y_{OHO,lys} \cdot f_{XU,E\_Bio,endoresp})} \cdot f_{XU,E\_Bio,endoresp} \tag{4.4}$$

The parameters are the OHOs growth yield from the model No. 2 and No. 3, $Y_{OHO,lys}$ = 0.625 mg CODx mg COD^{-1} (ASM 2d (Henze et al. 2000)), the endogenous respiration coefficient of model No. 1, $b_{OHO,endoresp}$ = 0.24 d^{-1} and the fraction of active biomass converted to endogenous residues in the endogenous respiration process of model No. 1, $f_{XU,E\_Bio,endoresp}$ = 0.20 mg CODx mg $CODx^{-1}$.

Table 4.1 : Overview of models used in this study. Model No. 1 Heterotrophs – CFSTR was primarily used for $b_{XU,E}$ estimation. Models No. 2 ASM2d extended – CFSTR and No. 3 ASM2d extended – AS-SSR were used to assess the impact of model choice on $b_{XU,E}$ estimation.

Model name	Purpose in this work and characteristics	Biokinetic model (and reference)	Biokinetic model parameter values (reference)	Reactor configuration model	Applied to data from
No. 1 Heterotrophs – CFSTR	**Estimation of $b_{XU,E}$.** Model No. 1 predicts Y_{obs} based on the simple biokinetic model and the simple reactor model of Spérandio et al. (2013). This model was used to assess $b_{XU,E}$ in general.	Synthesis of heterotrophs model of ASM1 (growth) and ASM3 (decay) + $X_{U,E}$ decay (Spérandio et al., 2013)	From Spérandio et al. (2013)	Fully mixed	Chon et al. (2011), Huang et al. (2014), Novak et al. (2007), Yagci et al. (2015)
No. 2 ASM2d extended – CFSTR	**Assess the impact of a complex biokinetic model on $b_{XU,E}$ estimation.** Model No. 2 was used to assess the impact of model complexity to $b_{XU,E}$ estimations, using a more complex biokinetic model (ASM2d) in combination with a simple reactor model.	ASM2d + $X_{U,E}$ decay (Henze et al., 2000)	From Henze et al. (2000)	Fully mixed	Chon et al. (2011)
No. 3 ASM2d extended – AS-SSR	**Assess the impact of a complex reactor configuration model on $b_{XU,E}$ estimation.** Model No. 3 with the more complex reactor configuration was used to assess the impact of the detailed reactor configuration model on $b_{XU,E}$.	ASM2d + $X_{U,E}$ decay (Henze et al., 2000)	From Henze et al. (2000)	Experimental reactor configuration	Chon et al. (2011)

Table 4.2 : Overview of selected biokinetic processes that are included in the three different models (Table 4.1). Only the decay process of ordinary heterotrophs (X_{OHO}) and of the endogenous residues ($X_{U,E}$) are displayed in this table 4.2. Model No. 1 Heterotrophs - CFSTR describes the dynamic processes behind Eq (2). Models No. 2 ASM2d extended - CFSTR and No. 3 ASM2d extended – AS-SSR describe the process according to ASM2d (Henze et al., 2000) with extension of the process of decay of endogenous residues that was added to ASM2d in this study. The parameter values indicate the values that were used in the simulations, the theoretical equivalent parameter values indicate the values that would have given equivalent mass loss behaviour for the endogenous respiration model and the death-regeneration model (see eq. (4.3) and eq. (4.4)).

Model name	Decay model type	Process\State variable	X_{OHO}	$X_{U,E}$	XC_B	Process Rate Expression	Parameter values	Theoretical equivalent parameter values
No. 1 Heterotrophs - CFSTR	Endogenous respiration	Endogenous respiration of heterotrophs	-1	$+f_{XU,E\_Bio,endoresp}$		$b_{OHO,endoresp} \cdot X_{OHO}$	$b_{OHO,endoresp}$ = 0.24 d^{-1} $f_{XU,E\_Bio,endoresp}$ = 0.20	
		Decay of endogenous residues		-1		$b_{XU,E} \cdot X_{U,E}$		
No. 2 ASM2d extended - CFSTR and No. 3 ASM2d extended – AS-SSR	Death - regeneration	Lysis	-1	$+f_{XU,E\_Bio,lys}$	$(+1-f_{XU,E\_Bio,lys})$	$b_{OHO,lys} \cdot X_{OHO}$	$b_{OHO,lys}$ = 0.40 d^{-1} $f_{XU,E\_Bio,lys}$ = 0.10	$b_{OHO,lys}^{*}$ = 0.56 d^{-1} $f_{XU,E\_Bio,lys}^{*}$ = 0.09
		Decay of endogenous residues		-1	+1	$b_{XU,E} \cdot X_{U,E}$		

Description of experimental studies and plant configurations

Average Y_{obs} and SRT values were selected from each individual experimental condition of the studies listed in Table 4.3. The average Y_{obs} and SRT corresponded to close to steady-state operational periods only (proximity to steady-state was determined in terms of Y_{obs}, SRT, sludge concentration). These studies were conducted with mostly synthetic wastewaters mainly composed of soluble organic substrates. Non-biodegradable influent TSS were thus not significantly present in those influent. The parameters collected from these experimental studies and used to estimate $b_{XU,E}$ with model No. 1 are reported in Table 4.4.

Table 4.3 : Literature studies used in this work for the estimation of $b_{XU,E}$. SBR stands for sequencing batch reactor, SSR stands for side-stream reactor.

Aspects of the studies	Reference			
	Chon et al. (2011)	Huang et al. (2014)	Novak et al. (2007)	Yagci et al. (2015)
Control system	SBR followed by aerobic digester	SBR followed by anaerobic digester	SBR followed by aerobic digester	SBR only[a]
Experimental system	SBR connected to an SSR	SBR connected to an SSR	SBR connected to an SSR	SBR connected to an SSR
Independent variables examined in the reference study	Aeration of the SSR	Reactor configuration (control vs. SBR-SSR)	Wastewater load and sludge interchange rate	Increased iron concentrations (iron addition of 2.7-16.1 mg Fe L^{-1}) ; reactor filling time (5 minutes; 4 hours)

[a] Yagci et al. (2015) refer to an experimental system of Khanthongthip (2010) as the control system

Table 4.4 : Experimental conditions and corresponding parameters used for estimation of decay rates of endogenous residues. The iron concentration of the influent wastewater is reported where available.

Aspects of the studies	Reference				
	Chon et al. (2011)	Huang et al. (2014)	Novak et al. (2007)	Yagci et al. (2015)	
Number (#) and name of experimental conditions	#1 "Chon AS-SSR aerobic" #2 "Chon AS-SSR anaerobic" #3 control	#1 "Huang AS-SSR anaerobic" #2 control	#1 "Novak AS-SSR anaerobic"[d] #2 control[d]	#1 "Yagci – slow filling – Fe 2.7" #2 "Yagci – slow filling – Fe 5.4" #3 "Yagci – slow filling – Fe 10.7" #4 "Yagci – slow filling – Fe 16.1" #5 "Yagci – fast filling – Fe 2.7" #6 "Yagci – fast filling – Fe 5.4" #7 "Yagci – fast filling – Fe 10.7" #8 "Yagci – fast filling – Fe 16.1" #9 control[e]	
Y_{obs} (mg VSS mg COD^{-1})	#1: 0.127[a]; #2: 0.126[a]; #3: 0.213[a]	#1: 0.114; #2: 0.333	#1 : 0.11; #2: 0.28	#1: 0.23; #2: 0.20; #3: 0.13; #4: 0.05; #5: 0.21;	#6: 0.13; #7: 0.08; #8: 0.05; #9: 0.374
SRT (d)	#1: 63; #2: 74; #3: 21	#1: 175; #2: 36.3[c]	#1: 80; #2: 30	#1: 31[f]; #2: 31[f]; #3: 53[f]; #4: 135[f]; #5: 46[f];	#6: 72[f]; #7: 144[f]; #8: 196[f]; #9: 9.7[g]
$f_{XU,Inf}$ (-)	0.01[b]	0	0	0	
$S_{iron,influent}$ (mg Fe L^{-1})	unknown	unknown	unknown	#1: 7.7[h]; #2: 10.4[h]; #3: 15.7[h]; #4: 21.1[h]; #5: 7.7[h];	#6: 10.4[h]; #7: 15.7[h]; #8: 21.1[h]

[a] Y_{obs} were reported in Chon et al. 2011 in units mg TSS mg COD^{-1}. Y_{obs} in units of mg VSS mg COD^{-1} was calculated based on provided VSS and TSS data from the corresponding author upon request (C. Park).

[b] 15% of influent particulate COD was assumed to be unbiodegradable.

[c] calculated based on reported values and additional information provided by the corresponding author (R. Goel) (calculation was based on reported wastage flow rate, solids concentration from the anaerobic SSR and the volume of the SSR).

[d] data from the first experimental phase in Novak et al. (2007) was used.

[e] Yagci et al. (2015) refer to an experimental system of Khanthongthip (2010) as the control system

[f] SRT was calculated based on reported information (Y_{obs}, COD load, HRT of the SSR, solids concentration in the reactor and the accumulation rate of solids in the reactors, the sludge wasting control and the sludge interchange control).

[g] The SRT was calculated based on the description in Khanthongthip (2010). The mass of solids in the reactor was calculated from reported sludge concentration and reactor volume. The sludge removal rate was calculated by the reported effluent solids concentration and effluent flow rate, the assumed sludge removal rate by sampling and the assumed sludge wastage mode (sludge wastage mode: removing $1/10^{th}$ of the settled solids, similar to the systems that were operated in parallel to the "control" reactor in Khanthongthip (2010)).

[h] calculated based on the iron dosage indicated in Yagci et al. (2015) and an assumed iron concentration in the water used for artificial wastewater preparation (5 mg Fe L^{-1} for groundwater (MWH, 2012)).

Sensitivity analysis

A sensitivity analysis was conducted to assess the feasibility of estimating $b_{XU,E}$ based on Y_{obs} values from the experiments of Chon et al. (2011), Huang et al. (2014), Novak et al. (2007) and Yagci et al. (2015) (Table 4.3, Table 4.4). The sensitivity analysis was based on model No. 1 (Table 4.1, eq. (4.2)). The parameter values for eq. (4.2) were chosen according to Spérandio et al. (2013): Y_{OHO} = 0.666 mg CODx mg COD, b_{OHO} = 0.24 d^{-1}, $f_{XU,E\_Bio,endoresp}$ = 0.20 mg CODx mg $CODx^{-1}$, $i_{COD,XOHO}$ = 1.42 mg CODx mg VSS^{-1}, $i_{COD,XU,E}$ = 1.55 mg CODx mg VSS^{-1}. $S_{U,Inf}$ and $f_{XU,Inf}$ were set to zero which corresponds close to observations in the considered experimental datasets (with exception of $f_{XU,Inf}$ = 0.01 for Chon et al. (2011) (Table 4.4)). Relative-relative sensitivity functions were determined according to Reichert (1994) for the independent parameters Y_{OHO}, b_{OHO}, $b_{XU,E}$, $f_{XU,E\_Bio,endoresp}$, varying one independent parameter (e.g. b_{OHO}) by a relatively small step, e.g., by 0.1% (Gujer 2008). The relative change in Y_{obs} (that is caused by the variation of the independent parameter) is then quantified. The relative-relative sensitivity is calculated as the ratio of the relative change in Y_{obs} (e.g. Y_{obs} (100.1% · b_{OHO}) - Y_{obs} (b_{OHO})) over the relative change in the parameter (0.1%). The relative-relative sensitivity function approximates how much Y_{obs} changes relatively to its normal value when a model parameter (e.g. b_{OHO}) changes by 100%.

Results

Sensitivity of the sludge yield towards endogenous residues decay

Model No. 1 does not predict correctly the values of Y_{obs} reported in the literature for AS-SSR systems (Fig. 4.1 a). Specifically model No. 1 underestimates Y_{obs} for SRTs less than 50 d while it overestimates Y_{obs} for SRT greater than 100 d if a nil $b_{XU,E}$ value is considered (Fig. 4.1 a). This confirms that predicting $b_{XU,E}$ values cannot be achieved by matching the Y_{obs} values of the AS-SSRs directly. We thus based our approach to estimate the range of $b_{XU,E}$ values on fitting the relative Y_{obs} reduction measured experimentally to the relative Y_{obs} reduction predicted by the models.

The sensitivity of Y_{obs} on Y_{OHO}, $b_{XU,E}$, b_{OHO} and $f_{XU,E\_Bio,endoresp}$ was determined using model No. 1 (Fig. 4.1 b). The relative-relative sensitivity function approximates how much Y_{obs} changes relatively to its normal value when a model parameter (e.g. b_{OHO}) changes by 100%.

At an SRT of 30 days, a system with a typical $b_{XU,E}$ of 0.007 d^{-1} shows 10% lower Y_{obs} than a system without $X_{U,E}$ decay (Fig. 4.1 b). For SRTs greater than 80 days, Y_{obs} is more sensitive to a relative change of $b_{XU,E}$ than to a relative change of b_{OHO} (Fig. 4.1 b). In contrast, the influence of Y_{OHO} and $f_{XU,E\_Bio,endoresp}$ on Y_{obs} remains significant even at high SRTs (Fig. 4.1 b).

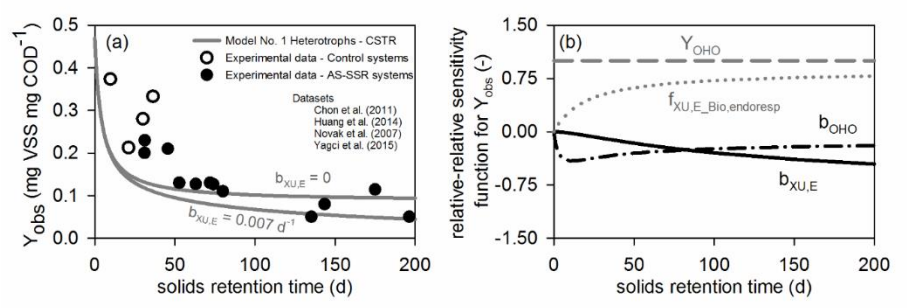


Fig. 4.1 (a) Experimental data and model of observed yield (Y_{obs}) and solids retention time. (b) Sensitivity function of Y_{obs} as function of four model parameters. (a) data points: from control activated sludge systems and from activated sludge – side-stream reactor (AS-SSR) systems reported in the literature (Chon et al. (2011); Huang et al. (2014); Novak et al. (2007); Yagci et al. (2015)). Models: Model No. 1 Heterotrophs – CFSTR (i.e. analytical model for Y_{obs} as function of SRT and decay rate of endogenous residues ($b_{XU,E}$)). (b) relative – relative sensitivity function for Y_{obs} dependent on the ordinary heterotrophs growth yield and decay rate (Y_{OHO}, b_{OHO}), fraction of endogenous residues in ordinary heterotrophs ($f_{XU,E\_Bio,endoresp}$) and $b_{XU,E}$. Relative - relative sensitivity analysis is also based on model No. 1.

Values of $b_{XU,E}$ estimated for AS-SSR systems with model No. 1

The values of $b_{XU,E}$ estimated from model No. 1 are greater than zero and vary over a range 0.001 to 0.24 d^{-1} (Fig. 4.2). A single $b_{XU,E}$ value was estimated for each of the 12 experimental conditions defined by Chon et al. (2011), Huang et al. (2014), Novak et al. (2007) and Yagci et al. (2015) and summarized in Table 4.3 and Table 4.4. Predicted $b_{XU,E}$ values are greater than zero which supports the hypothesis that $X_{U,E}$ degradation occurs in AS-SSR systems. The median value of estimated $b_{XU,E}$ values is 0.014 d^{-1} and the 10% and 90% percentiles are 0.002 and 0.183 d^{-1}, respectively. The median of the predicted $b_{XU,E}$ values is between the greatest reference $b_{XU,E}$ value of 0.0235 d^{-1} (as reported by Menniti et al. (2012)) and the values of 0.0070-0.0075 d^{-1} (Ramdani et al. 2012a, Spérandio et al. 2013) (Fig. 4.2). The approach for $b_{XU,E}$ estimation by Menniti et al. (2012) resembled the approach used in our study, but the

biokinetic model and the reactor configuration used by Menniti et al. (2012) were more complex than those used in our study for the estimation of $b_{XU,E}$. The estimation of $b_{XU,E}$ by Spérandio et al. (2013) was a direct parameter estimation with model No. 1 without considering the performance of control systems. The 10% quantile (0.0020 d^{-1}) is similar to other reported values from batch digestion experiments by Martínez-García et al. (2016) (0.0010-0.0040 d^{-1}) and Habermacher et al. (2016) (0.0001-0.0030 d^{-1}). The values of $b_{XU,E}$ predicted with model No. 1 were compared to different factors that can influence sludge production (Fig. 4.3). A weak linear correlation exists between the solids retention time (SRT) and $b_{XU,E}$ ($b_{XU,E}$ increases by 0.008 d^{-1} when the SRT increases by 100 days but the general distribution of $b_{XU,E}$ is very poorly described by the SRT (R^2=0.13) (Fig. 4.3 a)). A strong linear correlation is measured between $b_{XU,E}$ and the iron concentration in the influent wastewater ($b_{XU,E}$ increases by 0.0012 – 0.0032 d^{-1} per mg iron L^{-1} in the influent and much of the variability in $b_{XU,E}$ is explained by the iron concentration (R^2=0.78-0.97) (Fig. 4.3 b)), based on the experimental data of Yagci et al. (2015).

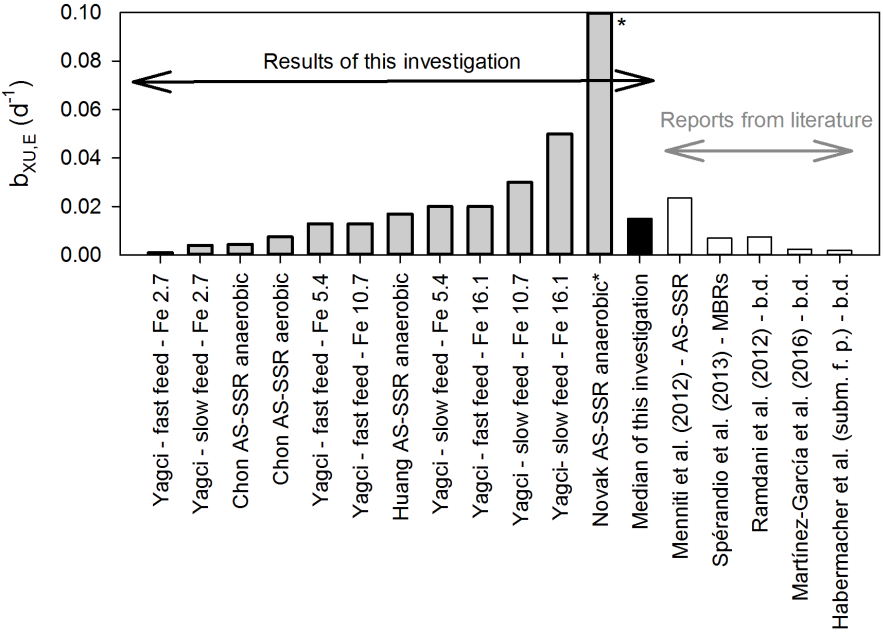


Fig. 4.2 Endogenous residues decay coefficients ($b_{XU,E}$) estimated from different reported AS-SSR systems. X-axis lists the different experiments from which $b_{XU,E}$ was estimated. Plot includes the median value of the $b_{XU,E}$ that were estimated in this study. *The study of Novak et al. (2007) yielded an infinite $b_{XU,E}$ thus a maximum value of $b_{XU,E}$ = 0.24 d^{-1} was assumed which corresponds to the estimated heterotrophs decay rate. All estimated and reported $b_{XU,E}$ values were determined under ambient temperatures. MBR stands for membrane bioreactor, b.d. stands for batch digestion.

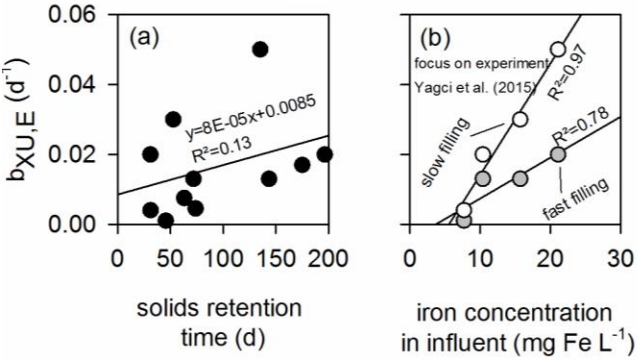


Fig. 4.3 (a) Values of endogenous residues decay rates ($b_{XU,E}$) as a function of the solid retention time (SRT) and (b) as a function of the influent iron concentration and reactor filling time. Estimations of $b_{XU,E}$ are based on model No. 1 Heterotrophs - CFSTR (i.e. an analytical model for Y_{obs} as function of SRT and $b_{XU,E}$). Plot (b) includes only the estimated $b_{XU,E}$ based on the data in Yagci et al. (2015). Linear trends, partly with slopes, intersects and coefficients of determination (R^2) are indicated. Data from Novak et al. (2007) was excluded due to uncertainty in exact $b_{XU,E}$ estimation.

Estimation of $b_{XU,E}$ with a more complex biokinetic model and the actual reactor configurations

Values of $b_{XU,E}$ were estimated with two additional models, No. 2 and No. 3, besides model No. 1, for the data of Chon et al. (2011) (Fig. 4.4 a). Model No. 2 comprises the complete ASM2d biokinetic model but a simplified reactor model, while model No. 3 contains the complete ASM2d biokinetic model and the actual reactor configuration. The values of $b_{XU,E}$ estimated using model No. 2 and No. 3 were greater than the values estimated by using model No. 1 (Fig. 4.4 a). The values of $b_{XU,E}$ estimated with model No. 2 were 84% and 111% greater than $b_{XU,E}$ estimated with model No. 1 for the AS-SSR aerobic and AS-SSR anaerobic system, respectively (Fig. 4.4 a). The $b_{XU,E}$ values predicted by Model No. 3 were 156% and 258% higher than the values of $b_{XU,E}$ estimated with model No. 1 for the AS-SSR aerobic and AS-SSR anaerobic system respectively (Fig. 4.4 a). The simulated sludge composition for the experimental system AS-SSR aerobic from Chon et al. (2011) was different between models No. 2 and No. 3 (Fig. 4.4 b). The simulations to determine the sludge composition (Fig. 4.4 b) were conducted with the experimental SRT of 63 days and $b_{XU,E}$ of 0.0192 d^{-1}, for both models,

No. 2 and No. 3 ($b_{XU,E}$ of 0.0192 d^{-1} was the estimated $b_{XU,E}$ for AS-SSR aerobic system with model No. 3 (Fig. 4.4 a)). With model No. 3 all sludge wastage was simulated to occur from the main reactor and none from the side-stream reactor. The simulation with model No. 3 shows that the main reactor of the AS-SSR systems contains a higher fraction of OHOs ($f_{OHO,AS}$ = 0.51 mg COD mg COD^{-1}) and a reduced fraction of inert or very slowly degradable residues ($X_{U,E}$ and $X_{U,Inf}$) compared to the side-stream reactor ($f_{OHO,SSR}$ = 0.31 mg COD mg COD^{-1}) (Fig. 4.4 b). The CFSTR according to model No. 2 has a sludge composition that is in between the one from the main reactor and the one from the side-stream reactor with model No. 3 (Fig. 4.4 b).

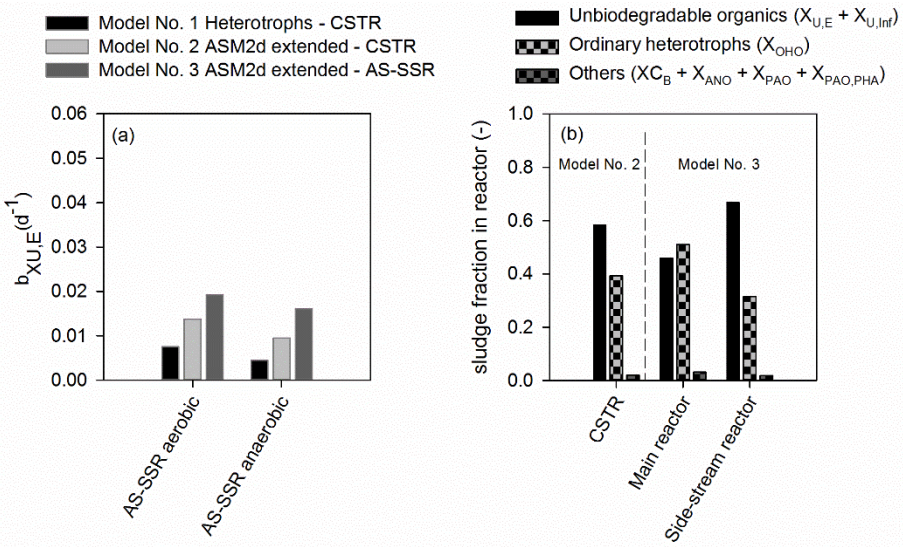


Fig. 4.4 Decay rate for endogenous residues ($b_{XU,E}$) estimated with three different models in plot a) and sludge composition by model No. 2 and No. 3 in plot b). Decay rates in a) were determined from the experimental dataset of Chon et al. (2011) on the activated sludge – side-stream reactor aerobic system (AS-SSR aerobic) and AS-SSR anaerobic system. Sludge composition in b) is for the AS-SSR aerobic system only. Model No. 1 is based on a simple biokinetic and a simple reactor model. Model No. 2 contains a more complex biokinetic model (ASM2d) but a simple reactor model (CFSTR). Model No. 3 contains the complex biokinetic model (ASM2d) and a more complex reactor configuration (AS-SSR). Symbols: $X_{U,E}$: Endogenous residues; $X_{U,Inf}$: Unbiodegradable influent particulate organics, X_{OHO}: Ordinary heterotrophic organisms; X_{CB}: Slowly biodegradable organic substrate; X_{ANO}: Autotrophic nitrifying organisms; X_{PAO}: Phosphorus accumulating organisms; $X_{PAO,PHA}$. Organic storage products of phosphorus accumulating organisms.

Discussion

Identifiability of $b_{XU,E}$ values from AS-SSR systems

Estimating the range of $b_{XU,E}$ values using the approach proposed in our study requires that (i) Y_{obs} is sensitive to $b_{XU,E}$ and that (ii) the degradation of $X_{U,E}$ is relevant for describing the Y_{obs} values in comparison to other biokinetic parameters (e.g. Y_{OHO}, b_{OHO} and $f_{XU,E\_Bio,endoresp}$). The sensitivity of Y_{obs} was estimated with model No. 1 and the sensitivity of Y_{obs} towards $b_{XU,E}$ was clearly demonstrated (Fig. 4.1 a and b). The difference between the predicted Y_{obs} for $b_{XU,E} > 0$

and for a zero $b_{XU,E}$ becomes especially significant (> 10%) when the SRT is larger than 30 days (Fig. 4.1 b). Reported experimental studies on AS-SSR systems that were selected from the literature for estimating $b_{XU,E}$ operated at SRTs greater than 30 days (Fig. 4.1 a). For these AS-SSR systems, the Y_{obs} was thus sensitive to the process of endogenous residue degradation and, in turn, to the value of $b_{XU,E}$. Considering endogenous residue decay is, thus, justified to explain low Y_{obs} values observed at high SRT since the decay of endogenous residues has been explicitly identified in batch digestion studies (Habermacher et al. 2016, Ramdani et al. 2012).

Nevertheless, Y_{obs} is also sensitive towards other parameters such as heterotrophic growth and decay parameters (Y_{OHO}, b_{OHO}, $f_{XU,E\_Bio,endoresp}$) (Fig. 4.1 b); thus it is important to have a good estimation of these other parameters. To account for the influence of these parameters on the predicted Y_{obs}, the approach for the estimation of $b_{XU,E}$ was developed to consider both the performances of the control and the AS-SSR systems. The control systems were mostly run in parallel of the AS-SSR systems, under similar conditions regarding influent composition and volumetric load, mixing and temperature as in the AS-SSR systems. It was thus assumed that the biokinetic characteristics in the AS-SSR systems are similar to the ones of the control systems. The Y_{obs} of the control systems were not well predicted with model No. 1 using default parameters (Fig. 4.1 a). This was a strong incentive to estimate $b_{XU,E}$ by taking into account the performance of the control systems rather than using model No. 1 with the corresponding typical parameters only. The applied approach for $b_{XU,E}$ estimation is described in section materials and methods, a similar approach was used by Menniti et al. (2012) for $b_{XU,E}$ estimation of an AS-SSR system.

The aim of this investigation was to determine the possibility of improving predictions of relative Y_{obs} decrease when a given MWRR plant at ordinary SRT is modified to operate with a much greater SRT (above 30 days) system of type AS-SSR. This should not be confused with the aim to improve numeric Y_{obs} predictions for a low SRT system, since the numeric Y_{obs} of the low SRT systems are not better predicted by considering $b_{XU,E}$ larger than zero (Fig. 4.1 a).

Estimated $b_{XU,E}$ in AS-SSR systems

All investigated data sets selected from the literature lead to estimation of $b_{XU,E}$ values larger than zero (Fig. 4.2). The main objective was to evaluate the range of $b_{XU,E}$ values, and especially

whether the predicted $b_{XU,E}$ values are greater than zero. The fact that all predicted $b_{XU,E}$ values were larger than zero suggests that the process of $X_{U,E}$ degradation should be included in the modeling of very long SRT systems such as the AS-SSR systems. The $b_{XU,E}$ values in Fig. 4.2 were estimated exclusively with model No. 1. The large distribution of $b_{XU,E}$ values observed in our study (10% and 90% quantiles of 0.002 – 0.183 d^{-1}) also suggests that several factors (i.e. wastewater and sludge characteristics or process conditions) influence $X_{U,E}$ degradation. Possible factors that influence $b_{XU,E}$ were reported by Yagci et al. (2015) that observed an impact of influent iron concentration and reactor filling time on Y_{obs}. Indeed, an increased iron concentration in the influent wastewater significantly increased the value of $b_{XU,E}$, considering the data from Yagci et al. (2015) (Fig. 4.3 b). Iron binds EPS in the sludge flocs under aerobic conditions and releases EPS under anaerobic conditions (Novak et al. 2003). Since EPS is part of the endogenous residue (Habermacher et al. 2016) it is possible that the endogenous residue becomes more biodegradable under anaerobic conditions with increasing content of floc bound iron. Additional factors could also affect the value of $b_{XU,E}$. One of these other factors is the reactor filling time, as highlighted by the estimated $b_{XU,E}$ values based on the data provided in Yagci et al. (2015) (Fig. 4.3 b).

SRT showed a positive correlation to $b_{XU,E}$ (R^2=0.13) (Fig. 4.3 a). Increased SRTs is often achieved by increasing the sludge concentration. The positive linear correlation between SRT and $b_{XU,E}$ could result from the effect of the active biomass concentration on the degradation of endogenous residues. If the active biomass has an effect on the degradation of endogenous residues, systems with high amount of active biomass (e.g. MBRs or AS-SSR systems) would then be associated with greater $b_{XU,E}$ values than systems with a relatively small amount of active biomass (e.g. batch digesters). Greater $b_{XU,E}$ values were reported for MBRs and AS-SSR systems ($b_{XU,E}$ = 0.0070 – 0.0235 d^{-1}) (Menniti et al. 2012, Spérandio et al. 2013) than for batch digestion tests ($b_{XU,E}$ = 0.0001 d^{-1} – 0.0040 d^{-1}) (Habermacher et al. 2016, Martínez-García et al. 2016). This supports the hypothesis that the degradation of $X_{U,E}$ is improved by the amount of active biomass in the system. Menniti et al. (2012) used a process rate for $X_{U,E}$ decay corresponding to a surface limited hydrolysis rate, thus including active biomass concentration as a driving factor for $X_{U,E}$ decay. Ramdani et al. (2012a) reported a large $b_{XU,E}$ value (0.0075 d^{-1}) measured during batch digestion tests, however, indicating that high $b_{XU,E}$ values can also be achieved in the absence of high amounts of active biomass. An influence of

active biomass on $b_{XU,E}$ could be expected but a more detailed investigation of this effect is required.

If further investigations show an importance of active biomass concentration on $b_{XU,E}$, then the SRTs in the SSRs must be shortened to maintain a certain amount of active biomass and ultimately improve the decay of $X_{U,E}$. Relatively short SRTs in SSRs were recently proposed by Park and Chon (2015) to decrease Y_{obs} of an AS-SSR system. If active biomass concentration is important for the degradation of $X_{U,E}$, then the rate expression for $X_{U,E}$ decay could be improved, e.g., by considering a surface limited hydrolysis rate expression.

Influence of model structure and parameter values on $b_{XU,E}$ estimation in AS-SSR systems

The models that were used in this study to estimate $b_{XU,E}$ values from the experimental data of Chon et al. (2011) resulted in different $b_{XU,E}$ estimates (Fig. 4.4 a). Nevertheless, the main conclusion is that endogenous residues undergo decay, as supported by the three models. All three models indeed predicted $b_{XU,E}$ values larger than zero (Fig. 4.4 a). The differences in $b_{XU,E}$ estimates using the three different models also indicate that $b_{XU,E}$ estimations from different reported experiments (Fig. 4.2) have to be compared with care. The comparability is limited because different model structures and parameter values were used in the $b_{XU,E}$ determination of Menniti et al. (2012), Spérandio et al. (2013), Ramdani et al. (2012), Martinez-Garcia et al. (2016) and Habermacher et al. (2016). The different results for $b_{XU,E}$ when determined with different models also indicate that values of $b_{XU,E}$ determined with a specific model and set of parameter values should only be applied for simulation tasks in combination with the specific model and parameter values.

The prediction of $b_{XU,E}$ using the model No. 2 is two times larger than the value of $b_{XU,E}$ predicted using the model No. 1. The higher value of $b_{XU,E}$ estimated with model No. 2 can result from the use of a more complex biokinetic model, from the use of decay parameter values that do not lead to equivalent mass loss in the endogenous respiration process (model No. 1) and the death-regeneration processes (models No. 2 and No. 3) (Table 4.2; Supplementary Information) and from the use of a numerical simulation tool. However, it was not evaluated what model describes the experimental systems from Chon et al. (2011) more accurately. Even

though ASM2d extended has the capacity to capture more biokinetic phenomena than the model No. 1, an inaccurate parameter choice with a complex model could lead to less accurate $b_{XU,E}$ estimation than using a simple model with accurate parameters.

If model No. 3 is used for $b_{XU,E}$ estimation instead of model No. 2 the estimation of $b_{XU,E}$ is further increased (Fig. 4.4 a). Model No. 3 includes the complex biokinetic model ASM2d and in addition the complex reactor configuration model for the control system (sequencing batch reactor followed by an aerobic digester) and for the AS-SSR system (sequencing batch reactor as the main reactor connected to the SSR for sludge interchange). Model No. 2 used a single continuous flow stirred tank reactor (CFSTR). Results in Fig. 4.4 a) showed that the reactor configuration considered in the models influences, in turn, the estimated $b_{XU,E}$ value. The sludge wastage explained the higher $b_{XU,E}$ values that are predicted with a model that considers the SSR. In fact, in model No. 3 the sludge composition in the main reactor varies from the one in the side-stream reactor (Fig. 4.4 b), f_{OHO} is 62% higher in the AS than in the SSR. Model No. 3 included wasting of sludge only from the main reactor. This was a simplification, based on the assumption that very long SRT systems would lose considerable amounts of sludge with the effluent. When sludge wasting is only considered to happen from the main reactor, rather fresh sludge from the main reactor is wasted and the older, partly digested sludge from the SSR is retained longer in the system. Wasting the fresh, active biomass and retaining older, slowly degradable material in the system can explain why an AS-SSR system with a given SRT has a higher sludge yield (Y_{obs}), compared to a fully mixed reactor with the same SRT (illustrated in Fig. S 4.1). Modeling the AS-SSR system with the complex reactor configuration rather than with a simplified CFSTR model thus leads to higher $b_{XU,E}$ estimate. The experimental data available from Chon et al. (2011) suggests that most sludge was wasted from the main reactor but also some from the SSR. Thus the precise reactor model would be an AS-SSR but with some sludge wastage from the SSR as well. It is recommended to consider the aspect of heterogeneous sludge composition and sludge wastage location for estimation of $b_{XU,E}$ in systems where sludge composition can be locally different.

Conclusions

- The degradation of endogenous residues ($X_{U,E}$) likely occurs in activated sludge – side-stream reactor (AS-SSR) systems. The predicted decay rates ($b_{XU,E}$) from twelve reported AS-SSR experimental datasets were consistently larger than zero (median = 0.014 d^{-1}), independent of the type of model used.
- Within the AS-SSR system, the localization of the sludge wastage (from the AS or SSR) influences the model-estimated value of $b_{XU,E}$. The composition of sludge in the AS reactor and SSR is different, with AS being "fresh" compared to the "old" sludge in the SSR (f_{OHO} 62% larger in the AS than in the SSR). This was observed in the mathematical modeling but can also be expected in actual treatment systems.
- The large variation of estimated $b_{XU,E}$ values indicated that wastewater characteristics (e.g., influent iron concentrations) or operating conditions (e.g., reactor filling time) influence the rate of $X_{U,E}$ degradation.

Acknowledgment

The authors would like to thank Chul Park and Ramesh Goel, the corresponding authors from the experimental studies of Chon et al. (2011) and Huang et al. (2014), for providing additional data about the experimental systems (measurements of VSS, TSS, reactor volumes and further precisions).

Supplementary Information

A) Model predictions of Y_{obs} with models No. 2 ASM2d extended – CFSTR and No. 3 ASM2d extended – AS-SSR

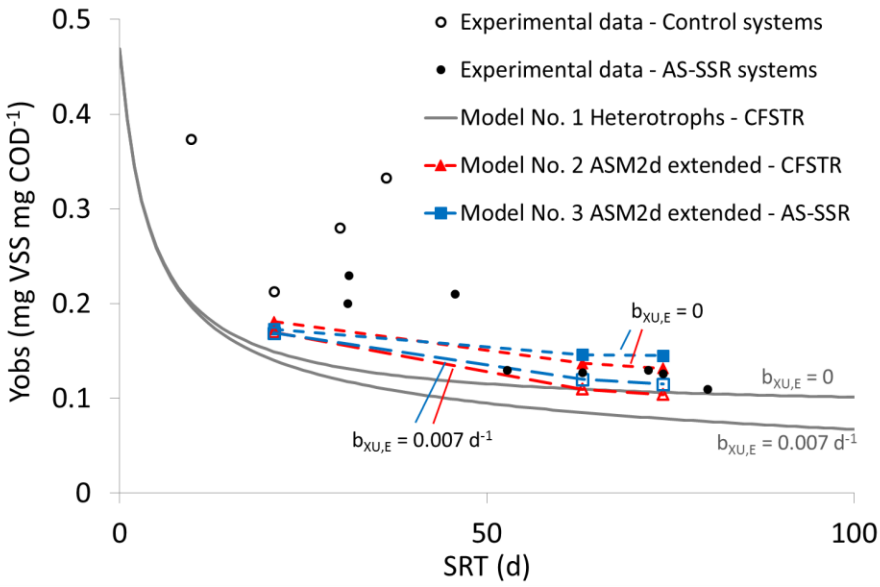


Fig. S 4.1 Observed sludge yield (Y_{obs}) as function of the solids retention time (SRT). The experimental data from Chon et al. (2011), Huang et al. (2014), Novak et al. (2007) and Yagci et al. (2015) is reported and the model values from model No. 1 Heterotrophs – CFSTR, all identical to Fig. 4.1 a), with exception of the SRT limitation at 100 days. The additional data to Fig. 4.1 a) are the model values from Model No. 2 ASM2d extended – CFSTR and Model No. 3 ASM2d extended AS-SSR. For model No. 2 and 3, only the precise Y_{obs} values for the SRTs at 21, 63 and 74 days were determined, the dashed lines between these values are linear interpolations.

Explanation of the models.

Model No. 1 predicted Y_{obs} as a function of SRT using eq. (4.2).

Model No. 2 simulated Y_{obs} for three experimental systems of Chon et al. (2011), the description of the experimental systems as follows:

- Control system with an activated sludge (AS) reactor (type SBR) and a conventional aerobic digester (system AS-Digester), experimental overall SRT = 21 days

- Experimental system with an activated sludge reactor (type SBR) and an aerobic side stream reactor (system AS-SSR aerobic), experimental overall SRT = 63 days
- Experimental system with an activated sludge reactor (type SBR) and an anaerobic side stream reactor (system AS-SSR anaerobic), experimental overall SRT = 74 days

Model No. 2 includes an influent model that corresponds to the experimental influent of Chon et al. (2011), the experimental reactor volume and the biokinetic model ASM2d (Henze et al. 2000), extended for degradation of the endogenous residues. The simplifications in this model No. 2 were i) the experimental reactor volumes of the activated sludge reactor and the connected digester were summed up and simulated as only one reactor (Table S 4.1) and ii) this one reactor was modelled as a continuously fed and aerated reactor that receives the average daily wastewater flow and substrate loads of the experimental systems of Chon et al. (2011). The individual reactor with constant volume was followed by a point settler, the sludge removal was hydraulic controlled to simulate the exact experimental SRTs of 21, 63 and 74 days, respectively. The simulations were run for a simulation time that exceeded three SRTs for reaching steady-state. At the end of the simulation the Y_{obs} could then be calculated as the simulated solids mass flow out of the reactor divided by the difference of total COD mass flow into the reactor and soluble COD mass flow out of the reactor. In comparison to model No. 2, model No. 3 included also a precise reactor and hydraulic flow model (Table S 4.1), corresponding to the physical system of Chon et al. (2011). In detail, the reactor volumes, hydraulic retention times and SBR cycle length were similar to the physical systems of Chon et al. (2011). To have the overall SRTs controlled with model No. 3 (at 21, 63 and 74 days, respectively) an algorithm was written into the "planwide.xlsx"-file of the SUMO ® simulations. For the control system, the SRT-controller calculated the sludge mass flowrate out of the digester and based on this calculated the necessary additional sludge mass flowrate out of the main reactor in order to reach the overall SRT of 21 days. In case of the AS-SSR systems in model No. 3, the only sludge transportation out of the system was simulated through the main reactor. Thus the SRT control algorithm only needed to calculate the sludge mass flowrate out of the main reactor in that case.

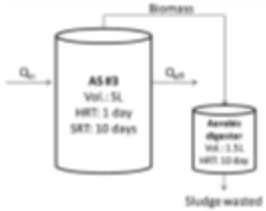
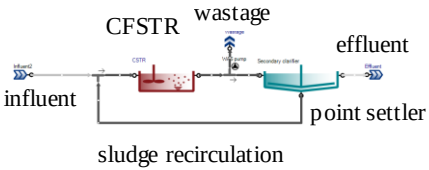
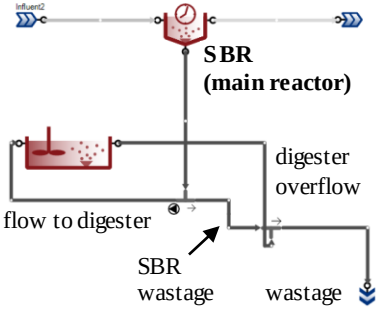
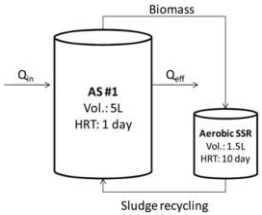
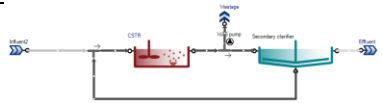
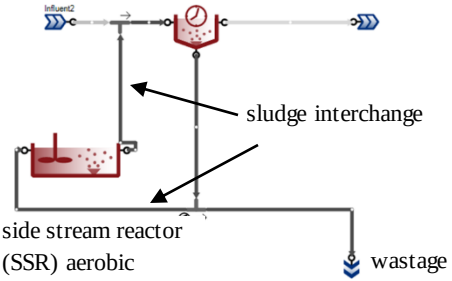
Description of the model results (Y_{obs} as function of the SRT).

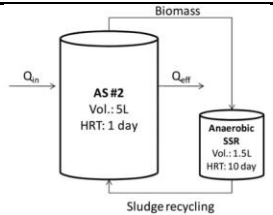
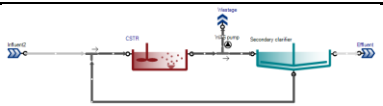
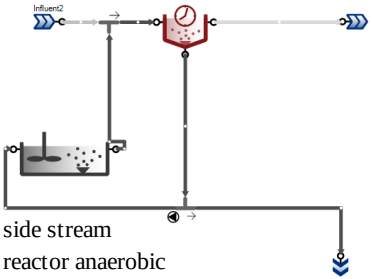
Model No. 2 and No. 3 predict higher Y_{obs} than model No. 1, for similar SRTs (Fig. S 4.1). Factors that contribute to higher Y_{obs} for model No. 2 and No. 3 are the decay parameters. As indicated in Table 4.2, the decay coefficient for heterotrophs, $b_{OHO,lys} = 0.40\ d^{-1}$, is lower in model No. 2 and No. 3 than the equivalent parameter based on model No. 1, $b_{OHO,lys}^{*} = 0.56\ d^{-1}$ (Table 4.2). In other words, the net decay of heterotrophs is slower in model No. 2 and No. 3 than in model No. 1. The other decay parameter is the fraction of endogenous residues contained in the active biomass, $f_{XU,E\_Bio,lys}$ in model No. 2 and No. 3. This parameter is higher in model No. 2 and No. 3 ($f_{XU,E\_Bio,lys} = 0.10$) than the equivalent parameter based on model No. 1 ($f_{XU,E\_Bio,lys}^{*} = 0.09$). In other words, the decay of one unit active biomass produces net more endogenous residues in model No. 2 and No. 3 than in model No. 1. One factor that has the opposite effect, i.e. increasing the predicted Y_{obs} with model No. 1 as compared to model No. 2 and No. 3, is the heterotrophic growth yield (Y_{OHO}). According to model No. 1, one mg of substrate COD is converted to 0.666 mg biomass COD. According to models No. 2 and No. 3 only 0.625 mg biomass COD are produced from one mg substrate COD. In the end the net effect of different factors leads to a higher prediction of Y_{obs} by models No. 2 and No. 3 as compared to model No. 1.

At the SRT of 21 days, model No. 2 predicts a slightly higher Y_{obs} than model No. 3 (Fig. S 4.1). The factor that can explain this is that in model No. 2 all sludge is in one reactor (Table S 4.1) and thus has a homogenous average sludge age and waste sludge is removed from the mixed liquor. In model No. 3 the aerobic digester contains old, partly digested sludge and most of the waste sludge is withdrawn from this reactor. Removing mostly old sludge but leaving fresh sludge in the system for stabilization (as in model No. 3) has the effect of decreasing Y_{obs}, as compared to removing sludge from a fully mixed reactor (as in model No. 2).

At the SRT of 63 and 74 days, model No. 3 predicts slightly higher Y_{obs} than model No. 2 (Fig. S 4.1). In model No. 3, for the AS-SSR systems all waste sludge is removed from the main reactor (Table S 4.1), thus rather fresh sludge is removed. Instead, the partly digested sludge from the side stream reactor is not wasted in model No. 3. Removing mostly fresh sludge but leaving old sludge in the system (as in model No. 3) has the effect of increasing Y_{obs}, as compared to removing sludge from a fully mixed reactor (as in model No. 2).

Table S 4.1 : Schemes of the physical systems, by Chon et al. (2011), and the implemented reactor models in the simulator SUMO ® according to model definition No. 2 and No. 3 (Table 4.1).

Name of the experi-mental system	Scheme of physical systems (Chon et al. 2011)	Reactor model of model No. 2 (illustrations created in SUMO ®)	Reactor model of model No. 3 (illustrations created in SUMO ®)
Control system AS-Digester	 *source: Chon et al. (2011)*		
AS-SSR aerobic	 *source: Chon et al. (2011)*		

AS-SSR anaerobic	 *source: Chon et al. (2011)*		 side stream reactor anaerobic

Chapter 5

General conclusions and outlook: Degradation of activated sludge under different redox conditions and degradability of endogenous residues for sludge reduction

Thesis chapter:

Habermacher, J., Morgenroth, E., Derlon, N.

Insights to sludge reduction processes

VSS and VSS fractions (cellular biomass, EPS) degraded faster under aerobic condition in side-stream digestion than under oxygen limited conditions (scarce and fermentation condition) (Chapter 2). VSS had a specific degradation rate under aerobic condition that was 2.2-4.1 times higher than under scarce aeration condition. Also the sludge reduction performance of a wastewater treatment system with side-stream reactor (SSR) was highest in general when the side-stream digestion was aerobic instead of oxygen limited. A main sludge reduction process in the activated sludge - side stream reactor (AS-SSR) system was thus shown to be degradation of VSS and VSS fractions (cellular biomass, EPS) in the SSR. Longer solids retention times (SRTs) in wastewater treatment systems led to lower excess sludge production yields (Y_{obs}). Lower Y_{obs} were achieved with longer SRTs regardless of the redox condition that was applied in the side-stream reactor. The important role of long SRT for excess sludge reduction is underlined because overall SRT has often received little attention in explaining sludge reduction in AS-SSR systems.

However, the beneficial increase of SRT to reduce excess sludge production reaches a limit if completely inert particulate material is accumulating. This is on the one hand because accumulation of such completely inert particulates would lead to operational problems, e.g. in terms of storage volume requirement and on the other hand to increase the retention time of only inert material does not lead to any additional sludge reduction. Traditional activated sludge models, such as ASM1-3 from IWA (Henze et al. 2000) assume the active biomass as biodegradable and the endogenous decay products as completely inert. According to such traditional models, the increase in SRT leads to an asymptotical decrease of the active biomass fraction in the sludge and inert residues show an asymptotical increase (Paul and Liu 2012). A very long SRT can thus be defined as the SRT beyond which excess sludge reduction becomes negligible according to traditional models. Values for very long SRT change with the type of treatment systems according to this definition (no more significant sludge reduction would be expected after 30 days of batch digestion (Ramdani et al. 2012a) but in membrane bioreactors Y_{obs} would still decrease to some small extent by increasing the SRT beyond 100 days (Spérandio et al. 2013)). Very long SRT leads to further excess sludge reduction if part of the

material that was traditionally considered as inert, such as endogenous residues ($X_{U,E}$), are biodegradable.

Endogenous residues from sludge produced on municipal wastewater were degradable at ambient temperature in absence of important amounts of active biomass. Similar conditions (ambient temperature and absence of important amounts of active biomass) can be found at small, decentralized wastewater treatment plants. The endogenous residues degradation rates ($b_{XU,E}$) for this sludge were 0.7-1.6·10^{-3} d^{-1} (Chapter 3). In AS-SSR systems an apparent degradation of endogenous residues was shown for twelve datasets from reported literature studies (Chapter 4). Previously only one precise value had been reported for AS-SSR systems. The estimated $b_{XU,E}$ showed a median of 14·10^{-3} d^{-1} and 10% and 90% percentiles of 2-183·10^{-3} d^{-1}. The sensitivity of $b_{XU,E}$ values estimation was quantified and $b_{XU,E}$ estimation in AS-SSR systems was shown to be affected substantially by the precision in sludge wastage modeling (Chapter 4). Regarding variability of $b_{XU,E}$ the batch experiments showed that sludge produced on synthetic wastewater and municipal wastewater degraded with similar rates (Chapter 3). The large variation of $b_{XU,E}$ values within the AS-SSR systems was also investigated for possible influence of wastewater characteristic or operational condition. A strong linear correlation for $b_{XU,E}$ with the influent iron concentration in combination with substrate injection duration was determined.

The degradability of EPS at very long SRT had not been investigated earlier, in contrast to endogenous residues. Our investigations showed that endogenous residues still have a substantial content of extracellular proteins and carbohydrates (300 mg g VSS^{-1}) and this EPS shows a similar degradability as endogenous residues. This conclusion is based on the variation of EPS during digestion of waste activated sludge. Most of the initial EPS mass of waste activate sludge was readily degradable (more than 50% of the initial EPS mass within the first eight days), so that endogenous residues were not consisting of EPS in majority. Nor was EPS completely depleted during digestion, because EPS were still present in endogenous residues after 260 days of digestion.

Increase of SRT in AS-SSR systems did not cause deterioration of effluent quality (as indicated by effluent VSS and effluent COD) nor deterioration of the sludge quality in the main reactor (indicated by SVI and filterability) in the AS-SSR system (Chapter 2).

Implementation of the process fundamentals at decentralized treatment plants for sludge reduction

Additional sludge reduction is expected to occur at very long SRTs because endogenous residues are biodegradable at ambient temperature. The prediction of exact sludge production rate is however challenging at very long SRTs because estimated degradation rates are quite variable.

The amount of sludge that is reduced with very long SRT due to the degradation of endogenous residues strongly depends on $b_{XU,E}$. The smaller $b_{XU,E}$ is, the higher the SRT needs to be to achieve a desired sludge reduction. The SRT can be increased by increasing the amount of sludge in a treatment system. For this the reactor volumes can be increased or the sludge concentration can be increased. However the increase of reactor volume or the increase of sludge concentration (e.g. with the help of membranes) leads to additional costs. Low values for $b_{XU,E}$ were found in batch digestion thus small scale decentralized treatment plants might have to use very large SRTs to have significant additional sludge reduction.

One way to achieve very long SRT is the application of a side-stream reactor (SSR). The SSR receives settled solids from the AS and allows thus to store more solids than a conventional AS. In addition, the increase of SRT in the AS-SSR system did not cause deterioration of effluent quality (as indicated by effluent VSS and effluent COD) nor deterioration of the sludge quality in the main reactor (indicated by SVI and filterability) (Chapter 2). This work showed that lower net sludge production can be achieved by exposing the sludge to aerobic condition rather than oxygen limited conditions. Operating the SSR aerobic rather than anaerobic is expected to bring additional benefits of reducing harmful gas emissions (H_2S, CH_4), requiring no sensitive methanogenic consortia for sludge reduction and providing local nitrification in the SSR. The expected drawbacks are a need for aeration in the SSR and thus a higher oxygen consumption. Also the pumping requirement increases slightly in a system including an SSR.

In general, there are side effects associated to the sludge reduction with very long SRT that will affect the treatment system. Nutrients and COD contained in the sludge will be released upon sludge degradation. In consequence the COD and nitrogen load to be oxidized increases, resulting in increased oxygen demand and the additional nitrification increases the alkalinity requirement. The released phosphorus by sludge degradation processes is found in the effluent in case no chemical precipitation is applied. Also if there is true inert material, for example hairs and fibers, this will accumulate in a very long SRT system. At full-scale AS-SSR treatment facilities true inerts are removed to large extents with mechanical devices (screen and hydrocyclone).

Outlook

Endogenous residues, composition and degradation. The estimated degradation rates of endogenous residues ($b_{XU,E}$) were variable across different experiments and also among reported values. Some observations suggest that $b_{XU,E}$ can be affected by wastewater, sludge or operational conditions. In Chapter 4 influent iron concentration and wastewater injection duration both influenced $b_{XU,E}$. Temperature dependency of $b_{XU,E}$ was shown in batch experiments by Ramdani et al. (2010) and Ramdani et al. (2012a). If a WWTP should be designed with a very low target observed yield (Y_{obs}) the large distribution of $b_{XU,E}$ leads to important design uncertainty. The design uncertainty could be reduced if factors that influence $b_{XU,E}$ were better understood and their effect on $b_{XU,E}$ quantified. It could even become possible to adjust process conditions to purposefully increase $b_{XU,E}$. This would not only be relevant for in-situ sludge reduction in WWTPs, but also for anaerobic digestion in order to enhance biogas production and to reduce the mass of anaerobic digestion residuals. On the one hand the composition of endogenous residues ($X_{U,E}$) could indicate possible degradation pathways for $X_{U,E}$ and how the degradability of $X_{U,E}$ could be influenced. On the other hand operational wastewater characteristics or process conditions that influence the degradation of $X_{U,E}$ can be determined experimentally.

Regarding $X_{U,E}$ composition, this thesis showed that there is a substantial content of EPS in $X_{U,E}$ (250–600 mg EPS g VSS^{-1}). The content of extracellular polysaccharides increased over digestion time relative to the content of extracellular proteins for two out of three sludges.

However the remaining part of $X_{U,E}$, that is not EPS but eventually dead biomass remains to be determined. It is proposed to investigate also the remaining part of $X_{U,E}$, first in terms of extracellular macromolecular classes (proteins, polysaccharides, lipids, and extracellular DNA) and then to determine specific molecules with a higher resolution (expected molecules in $X_{U,E}$ would be peptidoglycan from the cell walls, phospholipids from the cell membranes, carbohydrates). Such an analysis of $X_{U,E}$ composition can indicate on the one hand what is the important hydrolytic activity required to degrade $X_{U,E}$. Once the important hydrolytic activities are determined, different environments (aerobic, anaerobic, main reactors, digesters) could be investigated for the presence of the important hydrolytic activities. On the other hand $X_{U,E}$ hydrolysis could be studied by specific enzyme addition with the perspective to add this as a chemical for targeted, enhanced sludge reduction. In parallel to the organic molecules the inorganics in the sludge should be characterized because the inorganics can impact on activated sludge stability (Novak et al. 2003). Especially the divalent and trivalent cations (Mg^{2+}, Ca^{2+}, Fe^{3+}, Al^{3+}) should be analyzed in $X_{U,E}$ because they could also influence the degradability of $X_{U,E}$.

The operational conditions that were proposed to influence $b_{XU,E}$ are shear (Spérandio et al. 2013), pH (Martínez-García et al. 2016) or the oxygen concentration in aerobic dominated systems (Liu and Wang 2015) but these influences remain to be demonstrated, quantified and mechanistically clarified. If these conditions are confirmed to be relevant for $b_{XU,E}$ they could be considered in simulations. In addition the conditions of shear, pH and oxygen concentration can be influenced to some degree with a process design and thus an increase in $b_{XU,E}$ could become possible.

Sludge reduction remains an important topic at smallest scale of WWT to largest scale of WWT because the lower the sludge reduction is the more sludge residuals have to be treated, transported, and disposed. Thus the question of sludge degradation kinetic and extent, down to the level of the slowest biodegradable sludge fractions such as $X_{U,E}$, are of interest for the general WW industry.

Appendix

Appendix 1: Overview experiments

Experiment No.	Name	Time of experiment	Independent variables	Dependent variables
1	"4 systems SBR-SSR"	2011, January - September	Reactor configuration • SBR only • SBR-SSR • SBR-digester O_2 Redox condition • aerobic • anaerobic	Main: • Y_{obs} Secondary: • SVI • COD and solids in effluent
2	"Sludge digestion with iron addition"	2011, November-December	Iron dosage • no addition • addition Redox condition • aerobic • anaerobic	TSS, VSS
3	"3 systems SBR-SSR"	2012 July – 2014 July	Overall SRT • 25 d • 40 d • 80 d Redox condition • aerobic • scarce aeration • fermentation	Main: • Y_{obs} • ATP • EPS • Hydrol. enz. activity Secondary: • SVI • COD and solids in effluent

4	"pH effect on WAS degradability, in batch digestion"	2014 February	pH in batch-digesters • 5.5 • 7.5 • 8.5 (no control)	TSS, VSS
5	"pH effect on WAS degradability, in continuous digester (SSR) operation"	2014 March - April	pH in SSR-digesters	Main: • Y_{obs} Secondary: • SVI • COD and solids in SSR and effluents
6	"Influence of active biomass on degradation of endogenous residues"	2014 March - April	Ratio endogenous residues to active biomass concentration	Main: • TSS, VSS Secondary: • O.U.R.
7	"Composition and Degradability of active biomass, EPS and EPS fractions from 3 different sludges"	2014 May - 2015 May	Sludge origin • WAS from real WW • WAS from synth. WW • Batch Biomass	• TSS, VSS • ATP • EPS • EPS pools

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